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ABSTRACT

Perspectives, policy issues, and options for Congressional action that relate most directly to the development and implementation of alternatives to animal use in research and testing are addressed in this report. Testimonies and reports include those from the Office of Technology Assessment, the National Institute of Health, and the Food and Drug Administration. Discussion focused on the prospects for replacing, reducing, and refining animal use. Alternatives identified include: (1) continued but modified use of animals; (2) use of living systems particularly the in-vitro culture of cells, tissues and organs; (3) use of nonliving systems that mimic biological functions; and (4) computer programs. (ML)

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ALTERNATIVES TO ANIMAL USE IN RESEARCH AND TESTING

ED278547

HEARING

BEFORE THE
SUBCOMMITTEE ON
SCIENCE, RESEARCH AND TECHNOLOGY
OF THE
COMMITTEE ON
SCIENCE AND TECHNOLOGY
HOUSE OF REPRESENTATIVES
NINETY-NINTH CONGRESS
SECOND SESSION

MAY 6, 1986

[No. 130]

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Committee on Science and Technology

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ALTERNATIVES TO ANIMAL USE IN RESEARCH AND TESTING

TUESDAY, MAY 6, 1986

House of Representatives,
Committee on Science and Technology,
Subcommittee on Science, Research and Technology,
Washington, DC.

The subcommittee met, pursuant to call, at 10 a.m., in room 2325, Rayburn House Office Building, Hon. Doug Walgren (chairman of the subcommittee) presiding.

Mr. Walgren. Well, let me welcome you all to our hearing today. As you know this hearing will be focused on alternatives to animal use in research and testing, an area that has been of real interest to me and to other members of the committee for a number of years, ever since the Science, Research and Technology Subcommittee did some hearings on proposals for laboratory animal standards and the like, a number of years ago, and followed on with interest in the NIH bill in the Subcommittee of Energy and Commerce, that is involved in that.

As you all know, it is an area of developing possibilities, and one which we really want to keep thinking about, and keep on the forefront of our minds, because we don't want to miss those possibilities when they come along. The subject has had considerable attention for these several years, both because alternatives are economically attractive to the degree that they are less expensive for those who are involved in multiple testing; at the same time, some alternatives, we believe, have a certain degree of certainty about them that makes them more accurate than some animal testing in certain areas.

There also has been a developing and very real sensitivity in the public, and with signs of that certainly in the scientific community as well, that we want to be sure that we are as sensitive in our dealings with other living things as we possibly can be, and that there clearly must be a real justification for any of the pain and suffering, and consumption of animals that our society engages in. So there has been a gathering current of interest in finding alternatives for the use of animals in research, where possible.

We know that there are promising things that are happening. There have been reports that at the University of Michigan some people are experimenting with developing skins cells from volunteer donations, that can then be worked with in a way that can be used as a nonanimal, certainly, in that case a human being, for testing to show how skin reacts to toxins and the like.

Johns Hopkins, as we all know, has been working for several years with some funds from the private sector to try to develop an alternative to the Draize test. So these things are going on, and this committee wants to encourage that in any way that we can.

We have today, several witnesses who can bring us up to date on what the Federal Government is involved in, in attempting to develop and implement new methods that may not use animals, or use fewer animals, or cause less pain. There was, as you know, the recent OTA report "Alternatives to Animal Use in Research Testing and Education," which summarized the landscape as it seems to presently lie.

We have Dr. Gary Ellis, who was the project director for that report. He will start off with a summary of the ground that it covered.

We then will have a government panel, including Dr. James Willett, the Director of the Section on Biomedical Models and Materials Resources, that is a recently formed office in the National Institutes of Health, Division of Research Resources. He will be joined by Dr. David Rall, Director of National Institute of Environmental Health Services, which is also a a part of the NIH from Chapel Hill, NC; and Dr. Gerald Guest, Director, Center of Veterinary Medicine, for the Food and Drug Administration, who is accompanied by Elaine Esber, Director of the Office of Biologics Research and Review, Center for Drugs and Biologics, at the FDA.

Then after that we want to hear from Dr. Alan Goldberg, the director of the Johns Hopkins Center for Alternatives to Animal Testing, who will describe their program and give us some views from that perspective.

The thing I want to emphasize is that this should be an area of continuing discussion between the people that are involved in it. This committee is going to be very interested, and we see this as one of several hearings that we hope will keep the Government contribution focused on these developments as they happen within the Government and outside the Government; and certainly the Congress as a forum for the kinds of discussions that ought to take place, I think we can make a contribution in that area.

With that, why don't we proceed then, and call first Gary Ellis the project director of the OTA report.

Welcome to the committee and your written statements will be made a part of the record. Please feel free to underscore or outline in whatever way is most effective to focus our thoughts in our discussions. We appreciate your being here, and look forward to your testimony.

STATEMENT OF DR. GARY B. ELLIS, PROJECT DIRECTOR, BIOLOGICAL APPLICATIONS PROGRAM, OFFICE OF TECHNOLOGY ASSESSMENT

Dr. Ellis. Thank you, Mr. Chairman.

Mr. Walgren. I ought to mention that Congressman Boehlert, who is the ranking minority member on this committee, is over before Appropriations Subcommittee at this point, but will be joining us when he is free from that responsibility. So we will look forward to that.

Dr. Ellis.

Dr. ELLIS. Thank you. I am Gary Ellis of the Office of Technology Assessment, and I served as the project director for the recent OTA study to which you referred.

This morning I will first give a brief overview of the OTA report and then describe its principal findings. I will focus upon options for congressional action toward development and implementation of alternatives to animal use.

At the outset of the OTA study, it quickly became apparent that animal use in scientific experiments is not monolithic. There are three different broad areas of animal use: Research and biomedicine and behavior; testing for toxicity; and education in the life sciences.

The three are characterized by different procedures, different patterns of funding, different people carrying out the work, different motivations for animal use, and not surprisingly, different potential for alternatives to animal use. That really is the principal finding of the OTA report. The prospects for replacing, reducing, and refining animal are highly variable from application to application.

For most areas of scientific experimentation, totally replacing animal use with nonanimal methods, especially in the short term, is not likely. However, even if animals cannot be replaced in certain experiments, researchers can attempt to reduce the number used and also to minimize pain and distress.

Research, and to a lesser degree, testing, will continue to require live animals for observing complex interactions of cells, tissues and organs.

In testing, some whole animal methods are being replaced by nonanimal methods, as the new tests are validated. Federal regulatory agencies have recently indicted a willingness to accept data from alternative test methods.

In education, far fewer animals are used than in research and testing.

Computer simulations of living systems can replace or complement some animal use, particularly in education. However, use of animals is a prerequisite to the development of every more sophisticated computer simulations.

Reduction in numbers of animals used is also a principal alternative, but data currently available on animal use are very poor. Any estimate of animal use stands a rough approximation.

The best available data suggest a minimum of 17 to 22 million animals are used annually in the United States for experimentation. Rats and mice account for about 75 percent of those animals. Data are too poor to permit any definitive statement about trends in animal use through recent years.

Federal regulation of animal use in research and testing facilities occurs chiefly under the Animal Welfare Act, the Health Research Extension Act, rules of the EPA and FDA on good laboratory practices, and the policies of the Public Health Service and the NIH. The Animal Welfare Act is applied to dogs, cats, rabbits, guinea pigs, hamsters, and nonhuman primates, but not to rats and mice, the most common laboratory animals.

At the State level, most laws focus on matters such as procurement of animals rather than the actual conduct of experiments. Institutional and self-regulation occur by local review committees, that include lay members, and whose purview is expanding beyond traditional concerns of animal care to include aspects of animal use. The overwhelming majority of animal users are, or will soon be, subject to local committee oversight.

With that review of OTA findings as background, I will now address the development and implementation of alternatives.

In general terms, alternatives fall into one of four categories: First, the continued, but modified, use of animals, including a reduction in the number of animals used, improved experimental design and statistical analysis of results, and reduction of pain or experimental insult.

Second, the use of living systems, including invertebrates, microorganisms, and particularly, the in vitro culture of cells, tissues, and organs.

Third, the use of nonliving systems, such as epidemiologic data bases and chemical or physical systems that mimic biological functions.

And fourth, computer programs.

The process by which these and other alternatives become accepted practice in the research and testing communities consists of a sequence of four stages. Alternative methods are: One, developed through research; two, validated by independent measurements; three, gradually accepted by the scientific community; and four, implemented as they come to be relied upon or required.

Several alternatives are today in the validation or the implementation phase. For the most part these methods are based upon reductions and refinements in animal use. Approaches that replace the use of animals have generally not been completely validated and accepted; instead, these represent possibilities for the future.

Although the Federal Government has not directed funding toward the development of alternatives per se, it supports areas of basic research that can lead to alternative technologies. The areas of basic research most likely to contribute to alternatives include: cell, tissue, and organ-culture technology; research in animal health; understanding mechanisms of pain, pain control, and pain perception, and computer simulation of living systems.

Beyond support for basic research, how can the Federal Government stimulate development and implementation of alternative technologies?

OTA identified five options for congressional action, including the option of taking no additional action. I will run through these five options and then explain them.

First, Congress could require a new or existing Federal entity to coordinate the development, validation, and implementation of alternatives. This action would have great symbolic value within the scientific and animal welfare communities, and could accelerate the development of alternatives. A central clearinghouse for the development of alternatives could compile and maintain records of all federally funded research and development on alternatives. Information on R&D in the private sector would be a valuable component of the coordination effort, though it may prove difficult to obtain.

Coordinating activities could include symposia, workshops, newsletters, scholarships, grants, and the issuance of model protocols or guidelines. The coordinating body could monitor both public and private initiatives. Coordination could further involve identifying research areas likely to lead to new alternatives, and reviewing Federal support for these areas across agency lines. This latter responsibility might preclude housing this entity within an existing Federal agency involved in funding R&D on alternatives, to avoid either a real or apparent conflict of interest.

In February 1985, NIH created the Biological Models and Materials Resources Section within the NIH Division of Research Resources. With adequate funding, this office may serve as a focal point for the exchange of both biological materials and information about the use of model systems in biomedical research.

A second option, Congress could provide intramural and extramural Federal funding for the development of alternatives.

Development of alternatives in testing within the Federal Government is a natural offshoot of, and closely allied with toxicological research. The agencies most likely to produce alternatives in response to new Federal funding are the National Toxicology Program, the National Cancer Institute, the Food and Drug Administration, and Environmental Protection Agency, the Consumer Product Safety Commission, and the National Institute for Occupational Safety and Health.

To stimulate extramural R&D, granting agencies reviewing investigator-initiated applications could be required to assign priority to those that contain research with promise for development of new alternatives. This strategy would require sufficient flexibility to insure that valuable state-of-the-art scientific proposals that may not involve alternatives are not handicapped.

Postdoctoral training programs could be established, along the lines of NIH's National Research Service Awards, to insure a steady supply of young researchers schooled in traditional disciplines, ranging from molecular biology to animal behavior, disciplines with applications in the development of alternatives.

Financial incentives to private groups developing alternatives could take the form of tax incentives, perhaps, tax credits in addition to those already in place for R&D. Such groups could also be eligible for a new program analogous to the Small Business Innovation Research Program, that would target the development of alternatives.

A third option, Congress could encourage regulatory agencies to review existing testing guidelines and requirements and to substitute alternatives whenever scientifically feasible.

Through oversight or legislation, Congress could encourage or require Federal agencies to evaluate existing technologies and testing, to participate in their validation, to adopt them where appropriate, and to report to Congress on their progress in implementing alternatives, as the NIH is required to do by October 1, 1986, under the Health Research Extension Act of 1985. Such agency review would have to be a periodic or continuing effort, given the rapid advances in the state of the art.

The fourth option, Congress could ban procedures for which alternatives are available or give a Federal agency authority to ban procedures as valid alternatives become available. This option recognizes that prohibitions can be used to force technological change.

Prohibiting procedures for which scientifically acceptable alternatives are already available would accelerate the implementation of such alternatives. A ban could not only force implementation of existing alternatives, but, over time, help focus the development of new techniques.

A disadvantage of banning a specified procedure is that the replacement, or the process of developing one, may be even more politically unacceptable, for example, the in vitro culture of human fetal nerve cells. A prohibition also takes no account of the question of judging the scientific acceptability of an alternative.

In pursuing this option, it is important to appreciate that the swiftest adoption of alternatives may come about if regulatory agencies avoid mandating specific testing requirements. Requiring specified tests might actually serve as an inhibitor to the development and implementation of alternative methods. Greater flexibility is achieved when testing requirements are defined in a manner that allows judgment and encourages use of alternative methods. The adoption of alternatives might best be stimulated by regulatory requirement for evaluation of a potential toxic response, mutagenicity, for example, rather than requirement of specified test for mutagenicity.

A fifth option, Congress could take no additional action. If Congress takes no specific steps beyond its recent charge to NIH to establish a plan for the development of alternatives in biomedical research, the development of alternatives will continue to be a function of ethical, political, economic, and scientific factors.

That alternatives are being developed in the absence of direct legislation is best illustrated by research centers at the Rockefeller University and the Johns Hopkins University, funded by corporate and private donations. In addition, corporations are undertaking work in-house, or sponsoring it in universities, often in response to scientific, economic, animal welfare, and public relations concerns.

An uncertain pace of development marks the chief disadvantage of this option. Although alternatives may emerge, changing administrative, regulatory, and research priorities in both the public and private sectors will affect the rate of development.

Viewed from another perspective, this is an advantage. It permits researchers to respond to changing needs and priorities with minimal Federal interference.

In closing, I would like to note that beyond the development and implementation of alternative technologies, OTA identified five additional broad policy issues related to animal experimentation. Although these policy issues do not explicitly address either the development or implementation of alternative methods, they are inextricably linked to the replacement, reduction, and refinement of animal use.

The five additional policy areas are: One, disseminating information about experimentation; two, restricting animal use; three, counting animals used; four, establishing a minimum policy for intramural animal use within Federal agencies; and five, changing the implementation or amending the Animal Welfare Act.

I am providing material for the record to illustrate options for congressional action relative to each of these policy issues.

Mr. Chairman, I commend your efforts and those of the subcommittee in focusing attention in a constructive way on this often divisive issue. I thank you for the opportunity to present OTA's analysis of alternatives to animal use in research in testing.

[The prepared statement of Dr. Ellis, plus attachment follows:]

TESTIMONY OF GARY B. ELLIS
OFFICE OF TECHNOLOGY ASSESSMENT
U.S. CONGRESS
BEFORE THE SUBCOMMITTEE ON SCIENCE, RESEARCH, AND TECHNOLOGY
OF THE HOUSE COMMITTEE ON SCIENCE AND TECHNOLOGY

ALTERNATIVES TO ANIMAL USE IN RESEARCH AND TESTING

May 6, 1986

Thank you, Mr. Chairman. I am Gary Ellis of the Office of Technology Assessment, and I served as the project director for OTA's study of Alternatives to Animal Use in Research, Testing, and Education.

This morning, I will first give a brief overview of the OTA report and describe its principal findings. Then, I will focus upon policy issues and options for congressional action that relate most directly to development and implementation of alternatives to animal use in research and testing.

Summary and Findings of the OTA Report

At the outset of the OTA study, it quickly became apparent that there are three distinctly different areas of animal use: research in biomedicine and behavior, testing for toxicity, and education in the life sciences. The three are characterized by different procedures, different patterns of funding, different people carrying out the work, different motivations for animal use, and — not surprisingly — different potential for alternatives to animal use.

Analogously, a principal finding of the OTA report is that the prospects for replacing, reducing, and refining animal use are highly variable from discipline to discipline and application to application.

For most areas of scientific experimentation, totally replacing animal use with nonanimal methods, especially in the short term, is not likely. However, even if animals cannot be replaced in certain experiments, researchers can attempt to reduce the number used and also to minimize pain and distress.

Research, and to a lesser degree, testing, will continue to require live animals for observing complex interactions of cells, tissues, and organs. In testing, some whole animal methods are being replaced by nonanimal methods, as the new tests are validated. Federal regulatory agencies have recently indicated a willingness to accept data from alternative test methods. Chick embryo membranes, for example, are a promising alternative to rabbits' eyes for determining irritancy of chemical substances. Other test methods use cells, tissues, and organs in culture, as well as chemical and physical models. In education, far fewer animals are used than in research and testing.

Computer simulations of living systems can replace or complement some animal use, especially in education. However, use of animals is a prerequisite to the development of ever more sophisticated simulations. Computerized dissemination of research and testing results also could reduce some animal use.

Reduction in numbers of animals used is also a principal alternative, but data currently available on animal use are very poor. Any estimate of animal use stands as a rough approximation. The best available data suggest a minimum of 17 to 22 million animals are used annually in the United States for experimentation. Rats and mice account for about 75 percent of those animals. Data are too poor to permit any definitive statement about trends in animal use through recent years.

Ethical considerations are affecting the search for alternatives. At one end of a broad spectrum of views is the view that humans may use animals in any way. At the other end is the view that an animal has the right not to be used for any purpose not directly benefiting it. People throughout the spectrum find common ground in the principle of humane treatment, despite disagreement on exactly how the principle should be interpreted and applied.

Federal regulation of animal use in research and testing facilities occurs chiefly under the Animal Welfare Act, the Health Research Extension Act, rules of the Environmental Protection Agency and the Food and Drug Administration on good laboratory practices, and the policies of the Public Health Service and the National Institutes of Health.[1] The Animal Welfare Act is applied to dogs, cats, rabbits, guinea pigs, hamsters, and nonhuman primates, but not to rats and mice, the most common laboratory animals. At the state level, most laws focus on matters such as procurement of animals rather than the actual conduct of experiments. Institutional and self-regulation occur via local review committees that include lay members and whose purview is expanding beyond traditional concerns of animal care to include aspects of animal use. The overwhelming majority of animal users are (or will soon be) subject to local committee oversight.

For this study, OTA defined animals as nonhuman vertebrates: mammals, birds, reptiles, amphibians, and fish. Other creatures customarily included as animals -- invertebrates such as insects and worms -- are excluded by this definition. OTA did not examine animal use in food production; harvesting organs, antibodies, and other biological products; and sport, entertainment, and companionship. Such purposes include numbers of animals generally estimated to be many multiples greater than the numbers used for experimentation.[2]

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[1] In 1985, Congress enacted three laws citing alternatives to animal use: the Health Professions Educational Assistance Amendments of 1985 (P.L. 99-129), the Health Research Extension Act of 1985 (P.L. 99-158), and the Food Security Act of 1985 (P.L. 99-198), which amended the Animal Welfare Act.

[2] An estimated 2 to 4 billion animals are used in food production every year. (Ninety percent of those are chickens.) In addition, Americans have approximately 75 million dogs and cats as household pets.

Development and Implementation of Alternatives

In general terms, alternatives fall into one of four categories: First, the continued, but modified, use of animals, including a reduction in the number of animals used, improved experimental design and statistical analysis of results, and reduction of pain or experimental insult. Second, the use of living systems, including invertebrates, microorganisms, and particularly the in vitro culture of cells, tissues, and organs. Third, the use of nonliving systems, such as epidemiologic databases and chemical or physical systems that mimic biological functions. And, fourth, computer programs.

The process by which these and other alternatives become accepted practice in the research and testing communities is a sequence of stages. Alternative methods are (i) developed through research, (ii) validated by independent measurements, (iii) gradually accepted by the scientific community, and (iv) implemented as they come to be relied upon or required. Several alternatives are today in the validation or implementation phase; for the most part these methods are based upon reductions and refinements in animal use. Approaches that replace the use of animals have generally not been completely validated and accepted; instead, these represent possibilities for the future.

Although the Federal Government has not directed funding toward the development of alternatives per se, it supports areas of basic research that can lead to alternative technologies. The areas of basic research most likely to contribute to alternatives include (i) cell-, tissue-, and organ-culture technology, (ii) animal health, (iii) understanding mechanisms of pain, pain control, and pain perception, and (iv) computer simulation of living systems.

Beyond support for basic research, how can the Federal Government stimulate development and implementation of alternative technologies?

OTA identified five options for congressional action -- including the option of taking no additional action.

> **Congress could require a new or existing Federal entity to coordinate the development, validation, and implementation of alternatives.**

Implementation of this option would have great symbolic value within the scientific and animal welfare communities and could accelerate the development of alternatives. A central clearinghouse for the development of alternatives could compile and maintain records of all federally funded research and development (R&D) on alternatives. Information on R&D in the private sector would be a valuable component of the coordination effort, though it may prove difficult to obtain.

Coordinating activities could include symposia, workshops, newsletters, scholarships, grants, and the issuance of model protocols or guidelines. The coordinating body could monitor both public and private initiatives. Coordination could further involve identifying research areas likely to lead to new alternatives and reviewing Federal support for those areas across agency lines. The latter responsibility might preclude housing this entity within an existing Federal agency involved in funding R&D on alternatives to avoid either a real or apparent conflict of interest.

In February 1985, the National Institutes of Health (NIH) created the Biological Models and Materials Resources Section within the NIH Division of Research Resources. With adequate funding, this office may serve as a focal point for the exchange of both biological materials and information about the use of model systems in biomedical research.

Congress could provide intramural and extramural Federal funding for the development of alternatives.

Development of alternatives in testing within the Federal Government is a natural offshoot of and closely allied with toxicological research. The agencies most likely to produce alternatives in response to new Federal funding are the National Cancer Institute, the Food and Drug Administration, the Environmental Protection Agency, the Consumer Product Safety Commission, and the National Institute for Occupational Safety and Health.

To stimulate extramural R&D, granting agencies reviewing investigator-initiated applications could be required to assign priority to those that contain research with promise for development of new alternatives. This strategy would require sufficient flexibility to ensure that valuable, state-of-the-art scientific proposals that may not involve alternatives are not handicapped. Postdoctoral training programs could be established, along the lines of NIH's National Research Service Awards, to ensure a steady supply of young researchers schooled in traditional disciplines, ranging from molecular biology to animal behavior, with applications in the development of alternatives.

Financial incentives to private groups developing alternatives could take the form of tax incentives -- perhaps tax credits in addition to those already in place for R&D. Such groups could also be eligible for a new program (analogous to the Small Business Innovation Research program) that would target the development of alternatives.

Congress could encourage regulatory agencies to review existing testing guidelines and requirements and to substitute alternatives whenever scientifically feasible.

Through oversight or legislation, Congress could encourage or require Federal agencies to evaluate existing alternatives in testing, to participate in their validation, to adopt them where appropriate, and to report to Congress on their progress in implementing alternatives, as the NIH is required to do by October 1, 1986 (Public Law 99-158). Such agency review would have to be a periodic or continuing effort, given rapid advances in the state of the art.

Some review of testing guidelines now occurs in keeping requirements up to date, although the purpose of that review is probably to improve the science rather than to protect animals per se. The costs of agency review should be moderate, entailing input from agency experts, comment from outside experts, and publication. If Federal laboratories were involved in the validation of alternative testing methods, additional costs would be incurred.

Congress could ban procedures for which alternatives are available, or give a Federal agency authority to ban procedures as valid alternatives become available.

This option recognizes that prohibitions can be used to force technological change. Prohibiting procedures for which scientifically acceptable alternatives are already available would accelerate the implementation of such alternatives. A ban could not only force implementation of existing alternatives, but, over time, help focus the development of new techniques.

A disadvantage of banning a specified procedure is that the replacement, or the process of developing one, may be even more politically unacceptable (e.g., the in vitro culture of human fetal nerve cells). A prohibition also takes no account of the question of judging the scientific acceptability of an alternative.

In pursuing this option, it is important to appreciate that the swiftest adoption of alternatives may come about if regulatory agencies avoid mandating specific testing requirements. Requiring specified tests might actually serve as a strong inhibitor to the development and implementation of alternative methods. Greater flexibility is achieved when testing requirements are defined in a manner that allows judgment and encourages use of alternate methods. The adoption of alternatives might best be stimulated by regulatory requirement for evaluation of a potential toxic response -- mutagenicity, for example -- rather than requirement of a specified test for mutagenicity.

Congress could take no further action.

If Congress takes no specific steps beyond its recent charge to NIH to establish a plan for the development of alternatives in biomedical research, the development of alternatives will continue to be a function of ethical, political, economic, and scientific factors.

That alternatives are being developed in the absence of direct legislation is best illustrated by research centers at the Rockefeller University and the Johns Hopkins University, funded by corporate and private donations. In addition, corporations are undertaking work in-house or sponsoring it in universities, often in response to scientific, economic, animal welfare, and public relations concerns.

An uncertain pace of development marks the chief disadvantage of this option. Although alternatives may emerge, changing administrative, regulatory, and research priorities in both the public and private sectors will affect the rate of development. Viewed from another perspective, this is an advantage: it gives researchers the latitude to exercise their own judgment in responding to changing needs and priorities.

In closing, I would like to note that beyond the development and implementation of alternative technologies, OTA identified five additional broad policy issues related to animal experimentation. Although these policy issues do not explicitly address either the development or implementation of alternative methods, they are inextricably linked to the replacement, reduction, and refinement of animal use. The five additional policy issues are:

- disseminating information about animal experimentation;
- restricting animal use;
- counting animals used;
- establishing a minimum policy for intramural animal use within Federal agencies; and
- changing the implementation of or amending the Animal Welfare Act.

I am providing material for the record (see Attachment 1) to illustrate options for congressional action relative to each of these policy issues.

Mr. Chairman, I commend the Subcommittee for focusing attention in a constructive way on this often divisive issue, and I thank you for the opportunity to present OTA's analysis of alternatives to animal use in research and testing.

Attachment 1

Policy Issues Related to Alternatives to Animal Use and Options for Congressional Action

Policy issue						
Using existing alternatives	Developing new alternatives	Disseminating information	Restricting animal use	Counting animals used	Establishing a Federal animal-use policy	Changing Animal Welfare Act
Options for congressional action						
Take no action Charge a Federal entity with coordinating the implementation of alternatives Encourage alternative methods in Federal testing requirements Ban procedures for which alternatives are available	Take no action Charge a Federal entity with coordinating the development of alternatives Fund development of alternatives	Take no action Mandate easy access to federally funded testing and research data Promote greater use of testing data submitted to Federal agencies Require literature searches Create new data bases Translate foreign literature into English	Take no action Restrict use of certain kinds of animals Restrict use of certain protocols Restrict acquisition of animals from certain sources License animal users for certain protocols and/or kinds of animals Prohibit animal use	Take no action Eliminate APHIS[a] census Correct inadequacies in present APHIS[a] reporting system Expand APHIS[a] census to include rats and mice Establish independent census	Take no action Establish intramural Federal policy of minimum standards	Take no action Eliminate funding for enforcement Increase funding for enforcement Amend to expand coverage to include experimentation Amend to realign enforcement authorit Amend to preempt State and local laws

[a] Animal and Plant Health Inspection Service

SOURCE: Office of Technology Assessment

Mr. **Walgren**. Thank you, Dr. Ellis. In your exploration of this subject, how would you characterize the rate of development, do you find that there is more interest than may have been in previous years; and can you tell us anything about how fast things are happening in this area?

Dr. **Ellis**. Most certainly there is greater interest in alternative technologies than in previous years. The rate of development varies among research, testing, and education. Although the fewest animals are used in education, the greatest present incorporation of alternatives has occurred in educational uses. In testing there has been——

Mr. **Walgren**. Why is that?

Dr. **Ellis**. Perhaps because the nature of animal use in education differs so fundamentally from the use in research and testing. In education, animals are not generally used to develop new knowledge. Animals are used, or have been used, to train students in techniques or principles of scientific thinking and these goals can largely be accomplished through nonanimal methods.

In testing, progress has been made in developing alternative methods because, for one, industry has an economic incentive to minimize animal use whenever it can. Animal use is very costly; it is a labor-intensive type of endeavor, and industry has a strong profit motive to decrease animal use. So the progress in testing has been measurable.

In research, again, probably because of the nature of the research enterprise, progress has been most slowly. It is likely, as I said, that total replacement of animal use in research will not occur in the foreseeable future.

Mr. **Walgren**. How broad is the move toward nonanimal models in education; I don't know whether there is a standard curriculum or something like that, but has there been a substantial decrease in the use of animals in that area over the last 10 years?

Dr. **Ellis**. We were surprised at all levels of education, how few animals are actually used today. I have no measurements of previous years, but I would surmise that it certainly has decreased.

The Association of American Medical Colleges in conjunction with OTA, conducted a survey, so now I am talking about use at advance levels and the training of medical students. The survey was conducted at 16 of the 127 accredited medical schools in the United States.

Different departments in the medical schools differ in what use they make of animals. As might be expected departments of physiology, surgery and, I believe, pharmacology, make the greatest use of animals in training medical students.

But of the 16 schools polled, 6 departments of physiology make no use of animals in training medical students. We were surprised that only 10 of 16 in that sample polled used animals in training medical students in departments of physiology.

At lower levels, and undergraduate, and even graduate school, animal use appears to have declined for education purposes. Instead of each student, or a group of two students using an animal, a class demonstration may be held. This dramatically reduces the number of animals used in a particular session.

New video technologies have a role here, where a very sophisticated computer simulation, a pictorial video simulation linked to a computer can lead a student through a dissection exercise, which formerly would have been done in hands-on way. So animals are spared here in education.

Mr. **WALGREN**. What can you tell us about how that can be maximized, what is causing 1 of the 10 schools that still used animals to continue to use them, as opposed to one of the ones that had moved to a different mechanism.

Dr. **ELLIS**. I think that the educators would say that they are able to reduce animal use to a point, but beyond that point, the quality of education training of the physicians or veterinarians would suffer. The responses that we received from the medical and veterinary medical schools indicated that there may be a point below which further reduction may not be able to occur without sacrificing quality of education.

Mr. **WALGREN**. But assuming the six schools have not sacrificed quality education, are the others doing it just because we have always done it that way; what is it that is the difference between those schools?

Dr. **ELLIS**. As you said, that is probably one element of it, the attitudes of some educators. They have always done it this way; this is the best way. They, perhaps, are not interested in replacing animals. It may be——

Mr. **WALGREN**. Are there commercial products that we could rely on being offered to these other medical schools; how could we encourage that to happen?

Dr. **ELLIS**. I can cite one product that we illustrated in the report, in a picture. This is a manikin developed at the New York State Medical College at Cornell, the name of it is resusci-dog, so this is a dog—I am sorry—this is a doll, not a dog; it looks like a dog—where students can train in cardiopulmonary resuscitation, and in the veterinary medical classes this has eliminated the use of some number of the dogs.

The first model, a prototype was very expensive, in the thousands. The inventor estimates that this can be produced for hundreds of dollars now. I would watch for the dissemination of this one particular item to veterinary schools around the country.

Mr. **WALGREN**. Has that occurred yet?

Dr. **ELLIS**. I am unaware of it. I can't speak——

Mr. **WALGREN**. I guess what I am wondering is if there is—I suppose you can have orphan products out there that——

Dr. **ELLIS**. Presumably there would be an economic incentive here because the purchase of one resusci-dog, although the initial cost may be high, it would be saving use of dogs in laboratories, which, again, is a very expensive process.

Mr. **WALGREN**. Any indication of any ways that should be being discussed as to how to encourage these kinds of methods—I am thinking particularly, supposedly there are some computer simulations of living systems; are there blocks to the spread of the use of this kind of thing that you see?

Dr. **ELLIS**. In education—if that is what you continue to refer to—in education it is difficult to identify any blocks except the attitudes of some educators who just are not interested in changing. To represent their viewpoint accurately, I believe they feel that this is the best way for students to learn.

Mr. **Walgren**. How about in any other areas? Do you have any feel for—even though it is more difficult to feel you have an alternative, I guess, other than in education, because education is designed to simply transfer a certain experience to another.

How about in testing?

Dr. **Ellis**. In testing there are perhaps a handful of statutes that are actually requiring animal use. Again, this is a small number of statutes; the two that come to mind, the Hazardous Substances Act, which is enforced by the Consumer Product Safety Commission, requires that LD-50 tests be done to rate the hazardousness of a substance. Another statute enforced by the Department of Transportation, the Hazardous——

Mr. **Walgren**. As a statute, as opposed to a regulation?

Dr. **Ellis**. I believe it is in the statutory language. It is very unusual to find this, that is why I am noting it.

Mr. **Walgren**. Who oversees the Consumer Product Safety, that is a separate statute by itself, is it a separate agency?

Dr. **Ellis**. That is right; that is an independent agency.

Mr. **Walgren**. Separate authorization?

Dr. **Ellis**. I believe only a small amount of animal testing is actually done to comply with this statute, but it is one statute that actually names an animal test and requires it.

Another, I believe this is a statute enforced by the Department of Transportation, the Hazardous Materials Transportation Act, the way in which certain hazardous materials that are transported, the way in which they are classified or rated as to their hazardousness, whether they are a class A or B poison, is through specified animal testing. So the number of statutes that actually specify animal use are few and far between, but there are some, and this is an area to which the committee might want to turn its intention.

Mr. **Walgren**. You mentioned in your testimony that there are several methods that are in the validation stage now, can you tell us more about those?

Dr. **Ellis**. In testing, I think Dr. Goldberg, the fourth witness today, would be best equipped to describe—the development of a battery of tests, several different tests that may be able to serve where the Draize eye-irritant test has served in the past. One other test that we focused on in our report is the use of the chick embryo, I should say the membrane surrounding the chick embryo. This is a complete organ it has blood vessels, the tissue will respond to a caustic substance, with tissue damage it can, in fact, recover from injury, and this may be a substitute, or at least may complement the use of rabbit eyes, one of the most objectionable procedures to rate the toxicity of substances.

Mr. **Walgren**. What do you find when you look at the efforts to validate that—there is a picture in the book and, obviously, somebody has done it—how long a process is this validation, and who is it that gives an effort in that direction?

Dr. **Ellis**. It is my understanding that the initial work which was in a laboratory at the Medical College of Pennsylvania, was funded by three, or four, or five, animal welfare groups. The work has shown to be promising. In addition to the animal welfare groups, I believe Colgate/Palmolive is supporting this work and, in fact, they may be working in-house now to validate this. The procedures involves taking substances that are of known toxicity and labeling them in a blind fashion so the researchers in the laboratory don't know what they have, and asking, perhaps, two or three laboratories to go through this procedure, and administering the toxic substance to the chick embryo, to the rabbit eye, and comparing the results; this is the process of validation of a new test.

Mr. **Walgren**. Is there any way that we can know what size of effort is being made in pursuit of that?

You mentioned in your testimony that at the end, that the private sector is somewhat involved, and some corporations are interested; is there any way that we can know, as public, how much of an effort is being made in those areas?

Dr. **Ellis**. I don't know of any accurate source of figures across the drug or cosmetic industry, for example, on how much is being spent by the companies in developing alternative technologies. I don't know of any source of that data.

Mr. **Walgren**. The only thing else is the Government help, so that you would then look at the Government and ask what is being done there?

Dr. **Ellis**. In fact, asking the same question of the Government, it is difficult to obtain an answer because, for instance, we went to the National Science Foundation at the outset of our study, and asked, do they support research that involves alternatives to animal use. The answer almost by reflex was no. And perhaps, it was our fault because we hadn't asked the proper question.

I was surprised at that answer, and I said well, I know that you are supporting research at laboratory X, Y, or Z, with investigators developing a computer program to simulate blood flow in the dog intestinal system. And they said, oh, yes, we support that; we support these grants at laboratory X, Y, and Z. And I said, oh, this is what I am looking for. Then the National Science Foundation came back with a very nice breakdown, which we published in the book, on their support for grants that could lead to nonanimal methods or adjuncts to animal methods.

So it is very difficult to—I guess, it is a semantic problem, or a difficulty in communications, to go through each of the Federal agencies and ask them, what is your level of support for alternative technologies, because in many cases the alternative technologies really are offshoots of basic research. Asking that question is almost like asking what is your basic research budget.

Mr. **Walgren**. Thank you.

Mr. Boehlert?

Mr. **Boehlert**. Thank you, Mr. Chairman. I have an opening statement, I ask unanimous consent it be inserted in the record.

Mr. **Walgren**. Without objection.

[The prepared statement of Mr. Boehlert follows:]

OPENING STATEMENT
HON. SHERWOOD BOEHLERT

SRT SUBCOMMITTEE HEARING ON
ANIMAL RESEARCH
MAY 6, 1986

MR. CHAIRMAN:

TODAY'S HEARING CONCERNS ONE OF THE MOST VEXING ISSUES FACING SCIENCE--THE PROPER PLACE FOR ANIMALS IN TESTING AND RESEARCH.

THERE APPEARS TO BE A GROWING CONSENSUS THAT THE USE OF ANIMALS SHOULD BE AS LIMITED AS POSSIBLE AND THAT MORE ATTENTION SHOULD BE PAID TO THE WELL-BEING OF THOSE ANIMALS THAT ARE REQUIRED.

INDEED, THE USE OF ANIMALS IN TESTING AND RESEARCH SEEMS TO BE DECLINING FOR A VARIETY OF SCIENTIFIC, ECONOMIC AND POLITICAL REASONS. GOVERNMENT LAWS AND REGULATIONS, OF COURSE, ARE AMONG THE FACTORS ACCOUNTING FOR THIS APPARENT DECLINE.

THE QUESTION BEFORE US TODAY IS: IS THERE NEED FOR FURTHER GOVERNMENT ACTION TO DEVELOP AND PROMOTE ALTERNATIVES TO THE USE OF ANIMALS IN RESEARCH AND TESTING?

THE RECENT STUDY BY THE OFFICE OF TECHNOLOGY ASSESSMENT (OTA), WHICH IS SERVING AS A ROAD MAP FOR THIS HEARING, OUTLINES NUMEROUS POLICY CHOICES FOR CONGRESS, INCLUDING SIMPLY TAKING NO ACTION.

TODAY'S WITNESSES OUGHT TO GIVE US A CLEAR DESCRIPTION OF THE CURRENT USE OF ANIMALS IN INDUSTRY, ACADEMIA AND GOVERNMENT. WITH THESE FACTS, THE NEXT LOGICAL QUESTION TO ANSWER IS: HAVE WE STRUCK THE PROPER BALANCE BETWEEN THE NEED TO PROTECT ANIMALS AND THE NEED TO PROTECT PEOPLE?

OUR GOAL IS CLEAR--TO HELP HUMANITY THROUGH HUMANE RESEARCH. WE'VE BEEN MAKING PROGRESS IN RECENT YEARS TOWARD ACHIEVING THAT GOAL. I LOOK FORWARD TO HEARING FROM TODAY'S WITNESSES ON JUST HOW FAR DOWN THAT PATH WE'VE COME.

THANK YOU.

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Chief, Biological Models and Materials Resources Section, Animal Resources Program, Division of Research Resources, National Institutes of Health, Bethesda, Maryland, February 1985 - present

Formal Training:	Hours
The Federal Budget Process (NIH), 1980	24
Legislative Operations Roundtable for Executives (OPM), 1980	40
Survey of Modern Management Concepts (NIH), 1980	48
Advanced Management Seminar (OPM), 1980	40
American Association for the Advancement of Science 5th Annual Colloquium on R&D Policy, 1981	16
Division of Research Grants Incidents Course (NIH), 1981	8
Grants Associates Seminar Series (NIH), 1980-1981	160

Mr. **Boehlert**. Dr. Ellis, as I understand the LD-50 test, you get a group of animals and you keep feeding that group of animals a substance until at least 50 percent of the group dies, or the sample dies; is that correct?

Dr. **Ellis**. That is generally correct.

Mr. **Boehlert**. That seems sort of archaic to me, and inhumane. Is there any scientific reason for continuing the LD-50 program?

Dr. **Ellis**. There may not be a good scientific reason for continuing a classic LD-50 program, a test which involves a large squad of animals, maybe 100 animals. It appears that the fact of the matter is that a modified LD-50 test, using maybe only 30 animals, where 100 animals were used, gives you just as good an answer, or just as good information as the larger more classical test.

The reasons for using an LD-50 test at all are that it is a crude measure, a relative measure of toxicity of a substance, that is presumably why it was written into law in several statutes where poisons had to be differentiated from one another at some crude level.

Mr. **Boehlert**. I agree with your choice of word "crude."

Dr. **Ellis**. I don't use it in a pejorative sense; I use it in a descriptive sense only. It is crude.

Mr. **Boehlert**. I am using it in a pejorative sense.

Are you familiar with H.R. 1877?

Dr. **Ellis**. Is that Congresswoman Boxer's bill?

Mr. **Boehlert**. That is right.

Dr. **Ellis**. Yes.

Mr. **Boehlert**. What is your position on that legislation?

Dr. **Ellis**. OTA takes no position on any particular piece of legislation.

You have put me in a difficult position. I can talk about the items raised in that bill, I guess, and——

Mr. **Boehlert**. Well, don't speak for OTA then; speak for yourself. You are more scholarly in this area than I am. I am trying to learn from someone as bright and perceptive as you are.

Dr. **Ellis**. Well, you are perhaps, more crafty then I am in getting me to speak on an issue I don't want to speak on.

I can restate what I said and answer your previous question, and this is relevant to the Boxer bill, I suppose. The need for the classical LD-50 test is probably not present anymore.

I am not certain if the language of that bill talks about the classical LD-50 test, or if it just specifies LD-50 test. That actually would be something that the author of the bill, and the committee——

Mr. **Walgren**. Would you yield?

Let me understand one thing and that is that in the classic test are there always 100 animals, or might there be 1,000 and you looking for 500 dead animals?

Dr. **Ellis**. No, it could be a large number. No, I picked the number 100.

Mr. **Walgren**. So the total number of animals involved varies depending on who is doing the testing and what they want to have as background?

Dr. **Ellis**. That is right. The point is that the smaller number of animals gives you just as good a data as the large number of animals.

Mr. **Walgren**. Now, you said, maybe a population of 30 might be better, are you still looking for 50 percent of the animals to die?

Dr. **Ellis**. That is correct; that is what the value that you are looking for is. The LD-50 is a number. It is an amount of the toxic substance that killed half the group, lethal dose for 50 percent of the group.

Mr. **Walgren**. But it might as well be LD-1, or something like that; it could be?

Dr. **Ellis**. You would then have different information. Depending on your purposes you might be interested in LD-10, I suppose.

Mr. **Boehlert**. Dr. Ellis, are you aware that the bill does seek nonanimal alternatives for testing, and that it would permit testing in those instances where the testing is justified and reasoning behind it is made public through something in the Federal Register?

Dr. **Ellis**. You are calling it to my attention; that seems a good language.

Mr. **Boehlert**. I will put you down in the plus column.

Dr. **Ellis**. OTA, again, has no position on Congresswoman Boxer's legislation.

Mr. **Boehlert**. I said you; I didn't say OTA.

Dr. Ellis, which of the options that you have outlined in your testimony would you recommend; you have given us some options, do you have a recommendation?

Dr. **Ellis**. OTA makes no recommendations. Let me talk, again, on some that seem as if they might be the most doable and achieve the greatest progress.

Mr. **Boehlert**. All right.

Dr. **Ellis**. I think that the direction to NIH—so now I am talking about biomedical research—the direction to NIH last fall in the Health Research Extension Act of 1985, to report to the Congress within, essentially, a year, on its plan for alternative technologies, is a good thing. Direction to EPA, direction to FDA, and what other agencies you may feel relevant to do this kind of reporting to the Congress within a specified time, seems like it would be very helpful.

It is helpful at two levels. One, it lets the Congress know what the agencies are doing. Two, it forces the agencies, just as it forced NIH to take stock and to focus their attention in-house on this issue. That seems to be something that is doable, and something that would benefit all parties.

Mr. **Boehlert**. What do you think are the primary obstacles in our search for alternatives?

Dr. **Ellis**. In basic research, I think the nature of the process is a fundamental obstacle. Research, whether it is with animals or not, involves mistakes, missteps, serendipity, unexpected results, at times the unanticipated result is as important or more important than the anticipated result. And to put researchers into a straightjacket, perhaps, with a total ban on animal use, would so dim the scope of our quest for knowledge—to sound grandiose—but the fact of the matter is it is true that this would be, as the OTA report said, an outright ban on all species for all purposes, could be dangerous; the consequences to the public health are so unknown, so speculative to this extreme course of action, that this course of action could be dangerous.

So, I am talking about the most extreme course of action would have the most extreme consequences.

Mr. **Boehlert**. You don't see the debate that is going on as all species, and all circumstances, do you; isn't the——

Dr. **Ellis**. No.

Mr. **Boehlert** [continuing]. Debate centering around the humane treatment of animals?

Dr. **Ellis**. That is correct. That is where it is really the middle ground in the debate, where there is the most chance for progress. I didn't mean to dwell the extremes. But the amelioration of pain in the experimental procedures; the monitoring of those humans who are using the animals is extremely important, we didn't go into it explicitly in the report, but the first time animal user, whether it is a graduate student, well, we can start as an undergraduate doing that independent research project, this is a very hazy area in terms of oversight, and this is something where attention could be focused with positive results.

Mr. **Boehlert**. Dr. Ellis, thank you very much.

Dr. **Ellis**. Thank you.

Mr. **Walgren**. Thank you, Mr. Boehlert.

You mentioned, Dr. Ellis, in the summary, on page 26, that no one Federal agency policy on animal care has all the characteristics needed to address all the issues adequately, and that combining certain aspects of each would produce an effective uniform Federal policy. Can you develop that a little bit, because it isn't really developed in the summary?

Dr. **Ellis**. Yes; we are talking about intramural animal use here. As we viewed the total use of animals in this country, about 10 percent of the animals are actually used, we think, within Federal agencies, so this is intramural use.

Most of the use, about 50 percent of that intramural use, is NIH. The other two large users are the Defense Department and the Veterans' Administration, so this is animal use within Federal facilities. So I am talking about the oversight of animal use within those facilities.

The policy that applies now to the Federal facilities, I believe, is that policy that was part of the Food Security Act of 1985, and if I seem to be waivering it is because it is not exactly clear how this applies within Federal agencies. The Food Security Act, which amended the Animal Welfare Act, which is enforced by the Department of Agriculture, set out new guidelines for animal use in some 1,200 institutions around the country that are required to register with the Department of Agriculture under the Animal Welfare Act.

It also said that Federal agencies should follow these same procedures and report them, not to the Department of Agriculture, as most institutions do, but the Federal agencies should report to the chief executive of the particular agency. And it does not appear that the Department of Agriculture then has the privilege to inspect Federal agencies or oversight of Federal agencies, that would be a very unusual situation for one department to inspect or enforce the regulations on other departments.

My interpretation of the law was unclear. If I sound unclear, that is why. But there may be room for a uniform policy for animal use within Federal agencies.

Mr. WALGREN. Could I ask you to, in a submission, to address the differences between NIH, FDA, Department of Defense, and Veterans' Administration, the major users, with respect to their policies and how adequately they address these areas, so we could get a pretty good comparison?

Dr. ELLIS. Yes; I would be pleased to do that.

Mr. WALGREN. You mentioned the lack of information availability, and the information banks, one had been tried and discontinued; is that correct?

Dr. ELLIS. That is correct.

Mr. WALGREN. And now there is another effort which may or may not get sufficient funding; can you develop that a little bit?

Dr. ELLIS. Well, I think everyone is interested to see what happens now. The Food Security Act specified that the National Agricultural Library in conjunction with the National Library of Medicine, should begin a data base, or make information available to investigators on alternatives to animal use.

The case study that we went through in an effort in the late 1970's, early 1980's, the laboratory animal data bank failed miserably. It failed principally because the users were not interested in it. There was no way for the users to judge the reliability of the data that were in the data base.

Any user who goes to a data base will want to know the data have been peer reviewed, or at least judged in some way, they weren't contributed by a man on the street, and this imposes a much greater cost then, and a delay in getting the data into the data bank. So that may be an unconquerable sort of feature of a data base.

We prescribe that if any effort like this was to be undertaken that a user survey, a very sophisticated survey, and an expensive one, would be a good investment at the outset to insure that once some sort of data base, whether it is colossal, or whether it is medium size, is something that people will actually use, that it has something that people want. It was our feeling that to create such a data base, which would be very expensive, without having assured oneself that there is a desire for it, would be a poor thing to do.

Mr. WALGREN. Where was that defunct information bank housed?

Dr. ELLIS. I believe that NIH did it under contract, at least at one point, with Battelle, I believe. The number of user hours were pitifully small over 8 years, perhaps, 91 hours, something like that.

Mr. WALGREN. But now another effort is anticipated in the agricultural——

Dr. ELLIS. A slightly different sort of effort. Instead of having actual raw data—I should contrast the different types of computerized data bases that could be made. One might have raw data, where an investigator with an idea or protocol would check to see if this work had been done, if the numbers were available, and that could obviate the need for using animals. That is the effort to which I referred, the laboratory animal databank.

The Department of Agriculture has been told to create an information service on methods, materials, that would help train investigators in using animals. So I don't believe the Department of Agriculture is going to get into the data business, but they will be disseminating information that could help investigators in other ways.

Mr. Walgren. Well, perhaps you could also give us a submission of how that might help investigators in other ways?

Dr. Ellis. I would be pleased to.

Mr. Walgren. If you could develop that for the record.

Mr. Boehlert. Mr. Chairman, before we lose Dr. Ellis—I don't get as much opportunity as I would like to listen to someone with your special background, so let me throw a curve ball at you, if I may, not directly related to this. Are you familiar with the Department of Agriculture's position on the facial branding of cows in line with the whole herd buy up?

Dr. Ellis. I am aware from what I read in the newspapers, and an inquiry or two from congressional staff on the topic, yes.

Mr. Boehlert. What was your initial reaction to that; was that much to do about nothing; or uninitiated people like me who are offended by that proposal, somewhat on solid ground?

Dr. Ellis. Well, I think that the facial branding is offensive to any sensitive person. The USDA—and this is my own opinion—the USDA had a need to mark cows in some way. It is my understanding that the only area of the cow that the USDA has jurisdiction over, at least for that particular use, is the face area. Now, whether——

Mr. Boehlert. What? Who has jurisdiction over the rest of the cow?

Dr. Ellis. It is my understanding—you look at me with a look of incredulity. It is my understanding that different portions of the cow are branded for different purposes, and that one area that no one else can brand is the face area.

I may be mistaken, but that was my understanding. That is why USDA went to the face at the outset——

Mr. Boehlert. Well, is the tail end under the jurisdiction of the Department of Defense?

Well, they have changed, as you probably know.

Dr. Ellis. That is correct.

Mr. Boehlert. They are permitting the freeze branding.

Dr. Ellis. I think they would have been wise to go to the freeze branding at first, although it is still branding on the face, it somehow appears less offensive.

Mr. Boehlert. What about the dye injection method for the ear, isn't that something that is workable?

Dr. Ellis. Ears are notorious for changing their shape, changing their appearance. It is a less good method, but it may have served its purpose. I am not in a position to judge.

In the laboratory, for example, where you also have to identify animals for different purposes, not because they are dairy cows, but because you want to know which animal is getting which treatment, ears are often punched, and 1 week later a punch hole looks very much like a little bite made by a cage mate, and the No. 1 mouse, has turned into a No. 2 mouse. So ears are less good, I guess——

Mr. **Boehlert**. Well, I was thinking about the dye injection. I did some research on this. I was just——

Dr. **Ellis**. The dye injection method——

Mr. **Boehlert**. I am not only concerned about the inhumane treatment for animals, but I was kind of concerned for farmers.

Dr. **Ellis**. I certainly am not a defendant of the facial branding. I am only trying to bring some discussion to it since you asked.

Mr. **Boehlert**. Thank you very much.

Mr. **Walgren**. If the gentleman would yield.

Let me understand why they picked the face, again—because as I understand it, they had a reason to mark cows?

Mr. **Boehlert**. It is a jurisdictional dispute.

Mr. **Walgren**. Well, except that violates me more than—I had never thought of it that way. I had not heard this before, and it seems to me to be really striking. They had to mark cows; and they asked somebody, how are we going to mark cows; and the only suggestions they received had to do with the face because that is their only jurisdiction?

Dr. **Ellis**. Again, this is only my understanding, but I believe that no one else is allowed to put a mark on the cow, on the dairy cow, in that area, and so that is their privileged area, that is why they went to the face first. I believe that is true.

Mr. **Walgren**. It is one thing when somebody chooses it as a method of choice because it has certain advantages from everyone's aspect, everyone's standpoint, but to think that they chose that because in some statutory, totally unrelated development, that traditionally they had an area of focus, and they decided to brand in the area of focus.

What if their area of focus was the pupil of the eye, would that mean that they would only brand on the pupil of the eye?

That really doesn't make any sense. Even the freeze branding, maybe there is a better method of marking someplace else on the cow, where it ought to be, where there are less, perhaps, less nerves. I don't know that, but I would suspect it.

To the best of your knowledge that is a sort of an accurate state of the affair?

Dr. **Ellis**. I believe that the face area was an area of exclusion of any other marks, and when the need came to mark cows they turned to the area that they knew could have no other marks. I don't endorse it. I think it was a public relations blunder. But I think I am accurately representing the reason why.

Mr. **Walgren**. But then you get a public relations blunder and the response is to step back from hot branding to freeze branding. Maybe freeze branding is pretty easy stuff; I don't know.

Mr. **Boehlert**. Relatively speaking, it is.

Mr. **Walgren**. Yes.

Mr. **Boehlert**. They did respond, incidentally, because I went down the Florida house of—well, we are getting way far afield—but I went down to the Florida House of Representatives with a model cow, and three staff people grabbed me and said, you can't bring that on the floor of the House of Representatives, it is not dignified. But I brought it on and explained it.

Thank you, Dr. Ellis.

Dr. **Ellis**. Thank you.

Mr. **Walgren**. Thank you, Dr. Ellis. We appreciate your report, and parts of that will be made part of the record, as seem appropriate. We appreciate the report and your contribution today.

Dr. **Ellis**. Thank you.

[See subcommittee files for report mentioned above.]

Mr. **Walgren**. Let's call the next panel then. James Willett the Director of Biomedical Models and Materials Resources Section; David Rall, Director of the National Institute of Environmental Health Services; and Gerald Guest, the Director for the Center for Veterinary Medicine; the last of the FDA, the first two of the NIH.

Gentlemen, welcome to our discussions, we appreciate your being here. Your written statements will be made part of the record. Please feel free to outline or present the points which you feel deserve to be underscored in whatever way is most effective.

Let's go through in the order in which I introduced you to the audience, and start with Dr. Willett.

STATEMENTS OF DR. JAMES D. WILLETT, DIRECTOR, BIOMEDICAL MODELS AND MATERIALS RESOURCE SECTION, NATIONAL INSTITUTES OF HEALTH; DR. DAVID P. RALL, DIRECTOR, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SERVICE, NIH, CHAPEL HILL, NC; AND DR. GERALD GUEST, DIRECTOR, CENTER FOR VETERINARY MEDICINE, FOOD AND DRUG ADMINISTRATION

Dr. **Willett**. Mr. Chairman, members of the subcommittee, I am Jim Willett, Chief of the Biological Models and Materials Resources Section, of the Division of Research Resources, NIH. I am pleased to have the opportunity to present to this subcommittee information on the history, purpose, and activities of the Biological Models and Materials and Resources Section.

The Division of Research Resources Biological Models and Materials Section is developing a focus for the NIH's activities in the exploration and development of nonmammalian models for biomedical research. The section was created in February 1985 as an integral unit of the Animal Resources Program with a budget of $1.1 million.

The mission of the section is to provide for the development and support of cell systems, lower organisms, and nonbiological systems as models for biomedical research, and to provide biological materials that serve as critically important resources to the biomedical community. The section is addressing the need to explore and support the utilization of nonmammalian models in biomedical research.

While it is possible to view nonmammalian and nonbiological models as alternatives to mammalian models, such systems are best viewed as essential components of the range of model systems need for the efficient and effective pursuit of new knowledge in biology and medicine.

Over the last 5 years the division received numerous requests from the research community for support of a variety of model systems and needed biomaterials all identified as important research resources. The model systems so identified included: lower organisms, in vitro cells and tissues, and nonbiological models such . mathematical and computer simulation.

In response to these requests the division began in 1981 an examination of the range of model systems used in research supported by the NIH. The results of this effort enabled the section to track the use of human subjects, mammals, lower vertebrates and other living systems in the research projects NIH supports.

We know from the data that the relative distribution of projects employing mammalian models, human subjects, and other types of biological systems, such as the invertebrates, microorganisms, cells and cell products, et cetera, in the NIH's research portfolio, has remained essentially unchanged since 1977.

Mr. Chairman, I have included this table for the record which summarizes these findings.

[The table follows:]

Research Materials Use Update, May 1986. Dr. James D. Willett

Humans as Research Subjects

Fiscal Year	Research Dollars (%T$)	Projects (%T)
1977	27.5	32.4
1978	26.8	31.2
1979	26.8	29.2
1980	25.0	28.9
1981	23.8	29.7
1982	23.2	31.5
1983	22.9	32.2
1984	22.9	32.6
1985	22.9	32.8

Laboratory Animals (Mammals) as research Subjects

Fiscal Year	Research Dollars (%T$)	Projects (%T)
1977	43.5	41.9
1978	44.0	42.5
1979	44.9	43.8
1980	45.0	44.2
1981	47.3	44.1
1982	48.1	43.5
1983	47.9	42.7
1984	48.5	42.7
1985	46.2	41.4

All "Other"* Research Subjects

Fiscal Year	Research Dollars (%T$)	Projects (%T)
1977	29.4	25.6
1978	29.3	26.3
1979	28.2	27.0
1980	29.8	26.9
1981	28.9	26.0
1982	28.7	25.0
1983	29.2	25.1
1984	28.5	24.7
1985	30.0	25.8

* "Other" includes invertebrates, non-mammalian vertebrates, bacteria, viruses, mathematical and computer simulations, etc.

05-01-86

REPORT: JDW138

DIVISION OF RESEARCH RESOURCES
RESEARCH MATERIALS USE IN EXTRAMURAL RESEARCH PROJECTS
NATIONAL INSTITUTES OF HEALTH ONLY

RESEARCH CLASSIFICATION	1982 NO. OF PROJECTS	1982 % OF PROJECTS	1983 NO. OF PROJECTS	1983 % OF PROJECTS	1984 NO. OF PROJECTS	1984 % OF PROJECTS	1985 NO. OF PROJECTS	1985 % OF PROJECTS
H	9,617	31.1	10,456	31.9	11,016	32.3	12,210	32.5
HI	31	.1	34	.1	41	.1	44	.1
HM	3,362	10.9	3,480	10.6	3,532	10.3	3,605	9.6
HMI	98	.3	84	.3	96	.3	101	.3
HMN	184	.6	160	.5	160	.5	163	.4
HMNI	13	.0	8	.0	5	.0	5	.0
HN	54	.2	64	.2	61	.2	58	.2
HNI	12	.0	5	.0	7	.0	5	.0
I	659	2.1	686	2.1	702	2.1	711	1.9
M	8,791	28.5	9,263	28.2	9,735	28.5	10,599	28.2
MI	271	.9	296	.9	341	1.0	357	.9
MN	640	2.1	678	2.1	655	1.9	701	1.9
MNI	84	.3	54	.2	55	.2	50	.1
N	753	2.4	765	2.3	777	2.3	827	2.2
NI	72	.2	72	.2	62	.2	64	.2
R	6,249	20.2	6,721	20.5	6,886	20.2	8,111	21.6
TOTAL	30,890		32,826		34,131		37,611	

NOTE: H = HUMANS
I = INVERTEBRATES
M = NON-HUMAN MAMMALS
N = NON-MAMMALIAN VERTEBRATES

Dr. **Willett**. With these results in hand the division began examining the need for a research models and materials development program. The objectives of this activity were to explore the opportunities and limitations to the use of lower organisms, tissues and cells and culture, and mathematical and computer simulations as models in biomedical research.

In 1983, Congress asked the NIH to report on the division's activities in this area. Mr. Chairman, I would like to provide a copy of this report for the record.

[The report follows:]

ATTACHMENT 2

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
Division of Research Resources

REPORT ON BIOMEDICAL RESEARCH MODEL DEVELOPMENT

Thomas E. Malone

Thomas E. Malone, Ph.D.
Deputy Director, NIH

February 1983

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
Division of Research Resources

REPORT ON BIOMEDICAL RESEARCH MODEL DEVELOPMENT

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REPORT ON BIOMEDICAL RESEARCH MODEL DEVELOPMENT

INTRODUCTION

In its report on the Fiscal Year 1983 budget for the Department of Health and Human Services, the Committee on Appropriations stated:

> "DRR has taken the lead in planning the development of a new activity in 1983 entitled Biomedical Research Model Development. This activity will ascertain whether there are alternatives to the use of laboratory animals which can result in more reliable, economical, and efficient models to be used in biomedical research. In 1983, this activity will consist of planning efforts through workshops and conferences aimed at understanding the problems and identifying areas of research most likely to benefit from the development of models and the areas of technology most likely to yield usable research models. The Committee welcomes this effort to find alternatives to the use of laboratory animals for research. A report on the results of this effort should be made to the Committee at next year's hearings. If a program design is proposed, it should include estimates of total funding required, how such funds would be administered, the criteria for allocating funds, and the amounts recommended for Fiscal Year 1984." (House Report No. 97-894, pages 55-56)

The following report has been prepared by the National Institutes of Health of the Department of Health and Human Services in response to this request.

BACKGROUND

The mission of the Division of Research Resources (DRR) is to identify, develop, and maintain those resources that guarantee the quality of the environment in which modern biomedical research is performed. The development of models is an important ongoing activity in most scientific disciplines. The Division is focusing on those developed and developing model systems with broad applicability to biomedical research. Models development, like the development of other research methodologies, is an integral part of this mission.

A model is defined as "a representation to show the structure or serve as a copy of something." In biomedical research methodologies, models are used to provide simple or manageable examples of complex biological processes. A good model accurately portrays the system it is intended to represent. Models vary in their complexity. For example, nonhuman primates may be the best models for studies of the behavioral effects of a drug, while the best model of the drug's biochemical effects may be a system involving cells or tissues in culture.

ISSUE

The development of a model is often essential to the understanding of complex biological phenomena. Some research activities can use simpler systems as models (lower organisms, tissues and cells in culture, or nonliving systems) when seeking to answer questions of universal biological processes. Simpler model systems often provide data which are expensive, difficult, or impossible to obtain by using higher animals as models. From a scientific perspective, it seems advisable to determine if any of these specific simpler systems has general applicability. Intensive interest focuses on such developments today because of their potential for accelerating research findings, for dealing with multiple variables, and for reducing the current costs of biomedical research.

It is important to recognize that the development of simpler research models is not oriented toward creating alternatives to the use of animals in research. Only rarely, outside the area of biological testing, can one replace a complex experimental system (e.g., an intact tumor-bearing animal) with a significantly simpler one (e.g., tumor cells in a laboratory dish) and still be able to pursue the same scientific question. Whenever the research objective is to gain new knowledge about the life processes of intact higher organisms in health, in disease, or under various experimental circumstances, there are no alternatives to the use of laboratory animals.

CURRENT AND PROPOSED ACTIVITIES

The purpose in initiating the Biomedical Research Model Development activity is to foster the development and evaluation of biomedically important research methodologies based on lower organisms and nonliving models. The use of such models is invaluable in many areas of NIH-supported research. For example, invertebrate model systems can be employed to study such diverse areas as basic aspects of vitamin metabolism, the control of enzyme biosynthesis, metal ion toxicology, and hepatocellular carcinoma. Projects currently underway are using insects and nematodes in basic studies of the biology, biochemistry, and genetics of aging. Cell and tissue culture-based biological assays are also used in studies of a variety of physiological phenomena.

The wide range of biomedical research activities involving models which differ phylogenetically, structurally, and conceptually is apparent from a recent inventory of the research methods and models employed in Public Health Service (PHS)-supported research projects. The inventory makes possible the identification of research models employing lower organisms, tissues/cells in culture, or nonliving systems; pools of individual investigators having established expertise in each of the areas of potential model development; and areas currently supported by several NIH Institutes which are appropriate for potential model development.

DRR has received an unsolicited proposal from the Assembly of Life Sciences of the National Academy of Sciences to sponsor a series of workshops to identify areas of research which will benefit from the development of new or improved models for biomedical research and to provide lists of research methods suited to investigations in these areas. Specific model systems currently in use which have general applicability will be identified, and the characteristics promoting the interdisciplinary utility of such systems established. This overview of existing research models will clearly define the limits and opportunities for their application to major biomedical research problems, as well as the areas of overlap between model systems, where these exist. Mechanisms for stimulating research in modeling essential to the development of new or improved methods for biomedical research will be suggested where such activity is considered to have potential for economic or scientific value.

The panel of experts who reviewed the proposal concluded that the concept presented has potential interest and value in relation to the research objectives of several NIH Institutes and other Federal agencies. Discussions are underway with the Director, NIH, and the Institute Directors on the advisability of adopting this approach to the issues. The Division expects to begin the workshops in the latter part of 1983 with their completion anticipated by the end of 1984. A report of the results should be published by early 1985.

A specific extramural program in Biomedical Research Model Development will be developed if the results of the workshops indicate that such an activity is both necessary and meritorious. Assuming the results are favorable, the Division will prepare estimates of total funding required, and the criteria for allocating and administering funds. Requests for grant applications could be issued as early as 1984, with initial awards being made in 1985 or early in 1986.

Dr. **Willett**. The 1983 report explained the purposes for NIH's initiation of the biomedical research model development activity, which was to foster the development and evaluation of biomedically important research methodologies that are based on lower organisms and nonliving models.

In this same year NIH expanded its evaluation through a contract with the National Academy of Sciences of the opportunities and limitations to the use of lower organisms, in vitro cells and tissues, and nonbiological approaches in developing models for biomedical research.

In March of 1985 NIH received the results of the academy's evaluation of modeling in biomedical research in a report entitled, "Models for Biomedical Research: A New Perspective."

Mr. Chairman, I would like to provide you with a copy of the report for inclusion in the hearing record.

Mr. **Walgren**. We appreciate that.

[The report, "Models for Biomedical Research: A New perspective," is available in the subcommittee files.]

Dr. **Willett**. In conducting the evaluation a case study approach was used, sampling the spectrum of biomedical research modeling. Five topics, each covering an important area of biomedical research, were selected for in-depth examination through a series of workshops.

In this manner the academy committee conducting the study examined and evaluated experimental models for the investigation of cellular immunology, regulation, learning, diseases and aging, and development. A sixth workshop was subsequently arranged to examine mathematical modeling in biomedical research.

In great measure the report deals with the the theoretical structure of general biology and reemphasizes the concept of unity and diversity so long held by biologists. From the breadth of topics covered during the course of the workshops held, it became more and more evident that in biology, as the models report states, "at every hierarchical level from molecules to ecosystems, common hardware, common programs, and common strategies are used to achieve diverse ends."

The Biological Models and Materials Resources Section has begun activities in response to four of the recommendations in the academy report. These recommendations are similar to requirements in the Research Extension Act, Public Law 99-158, in section 4. Further, the section is serving as a home for several important research resources.

We are fully supporting two important resources, the American Type Culture Collection and the Cell Culture Center at the Massachusetts Institute of Technology, and is shearing support for three additional resources whose primary support is with another NIH institute, these are the Repository for Human DNA Probes and Libraries, the National Diabetes Research Interchange, and the Caenorhabitis elgens Genetics Center.

This concludes my prepared statement, and I would attempt to answer any questions the subcommittee may have.

[The prepared statement of Dr. Willett follows:]

FOR RELEASE UPON DELIVERY

STATEMENT BY
JAMES D. WILLETT, PH.D.
CHIEF

BIOLOGICAL MODELS AND MATERIALS RESOURCES SECTION
ANIMAL RESOURCES PROGRAM
DIVISION OF RESEARCH RESOURCES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOR THE HEARINGS ON
ALTERNATIVES TO ANIMAL USE IN RESEARCH AND TESTING

BEFORE THIS
SUBCOMMITTEE ON SCIENCE, RESEARCH AND TECHNOLOGY
COMMITTEE ON SCIENCE AND TECHNOLOGY
U.S. HOUSE OF REPRESENTATIVES

May 6, 1986

Mr. Chairman, and members of the Subcommittee, I am Dr. James D. Willett, Chief of the Biological Models and Materials Resources Section of the Division of Research Resources, NIH. I am very pleased to have the opportunity to present to this Subcommittee information on the history, purpose and activities of the Biological Models and Materials Resources Section.

General Program Description

The Division of Research Resources Biological Models and Materials Resources Section is developing a focus for the National Institutes of Health's (NIH's) activities in the exploration and development of nonmammalian models for biomedical research. The Section was created in February of 1985 as an integral unit of the Animal Resources Program with a modest budget of $1.1 million. The mission of the new Section is to provide for the development and support of cell systems, lower organisms and nonbiological systems as models for biomedical research, and to provide biological materials which serve as critically important resources to the biomedical community. The Section is addressing the need to explore and support the utilization of nonmammalian models in biomedical research.

While it is possible to view nonmammalian and nonbiological models as alternatives to mammalian models, such systems are best viewed as essential components of the spectrum of model systems needed for the efficient and effective pursuit of new knowledge in biology and medicine.

Background

Over the last five years the Division received numerous requests from the research community for support of a variety of model systems and needed biomaterials identified by them as important research resources. The model systems included: lower organisms, in vitro cells and tissues, and nonbiological models such as mathematical and computer simulations.

In response to these requests the Division began, in 1981, an examination of the range of model systems used in research supported by the NIH. The results of this effort enable the Section to track the use of human subjects, mammals, lower vertebrates and other living systems in the research projects NIH supports.

We know from the data gathered that the relative distribution of projects employing mammalian models, human subjects and other types of biological systems (such as invertebrates, microorganisms, cells and cell products, etc.), as well as nonbiological model systems in NIH's research portfolio, has remained essentially unchanged since 1977.

irman, I have included a table for the record which summarizes these findings.

With these results in hand, the Division began examining the need for a research models and materials development program. The objectives of this activity were to explore the opportunities and limitations to the use of lower organisms, tissues and cells in culture, and mathematical and computer simulations as models in biomedical research.

In 1983, Congress asked the NIH to report on the Division's activities in this area.

Mr. Chairman, I have provided a copy of this report for the record.

The 1983 report explained the purpose for NIH's initiation of the Biomedical Research Model Development activity, which was to foster the development and evaluation of biomedically important research methodologies that are based on lower organisms and nonliving models.

In this same year NIH expanded its evaluation of the opportunities and limitations to the use of lower organisms, in vitro cells and tissues, and nonbiological approaches in developing models for biomedical research, through a contract with the National Academy of Sciences.

In March of 1985 the National Institutes of Health received the results of the Academy's evaluation of modeling in biomedical research in a report entitled, Models for Biomedical Research: A New Perspective.

Mr. Chairman, I'd like to provide you with a copy of the report for inclusion into the Hearing Record.

In conducting the evaluation a case study approach was used, sampling the spectrum of biomedical research modeling. Five topics, each covering an important area of biomedical research, were selected for in-depth examination through a series of workshops. In this manner the Academy committee conducting the study examined and evaluated experimental models for the investigation of cellular immunology, regulation, learning, diseases and aging, and development. A sixth workshop was subsequently arranged to examine mathematical modeling in biomedical research.

The synthesis of concepts presented during the workshops led to insights into modeling and information transfer in biological research that provides a conceptual framework for how models can be selected and used. In conducting the analysis of models the committee strove to answer three questions basic to the modeling process:

- How can information gained from studies of organisms simpler than humans, be used to expand our knowledge of human biology in normal and pathological states?

- What is the degree of confidence in the transfer of information gained from one species to studies of another--i.e., to what extent does a general biology (a structure analogous to theoretical physics that subsumes particular cases within general laws) exist, and to what degree have biologists succeeded in formulating it?

o How are the problems inherent in information transfer related to the levels of organization (e.g., molecules, cells, tissues, organs or organisms) of the phenomena under study?

In great measure the report deals with the theoretical structure of general biology and reemphasizes the concept of "unity in diversity" so long held by biologists. From the breadth of topics covered during the course of the workshops held, it became more and more evident that in biology, as the models report states, "at every hierarchical level from molecules to ecosystems, common hardware, common programs, and common strategies are used to achieve diverse ends."

Academy Recommendations

Contained within the report on Models for Biomedical Research: A New Perspective are eight recommendations to the NIH regarding modeling and model development in biomedical research. The report recommends that the NIH should:

1. support good research without taxonomic or phylogenetic bias including comparative and phylogenetic studies. Proposals for the study of invertebrates, lower vertebrates, microorganisms, cell and tissue culture systems, or mathematical approaches should be regarded as having the same potential relevance to biomedical research as proposals for work on systems that are phylogenetically more closely related to humans.

2. strive to make favorable systems available to the research community by: providing support to supply organisms for research, maintaining stock centers for mutant strains and cell lines, facilitating access to computer programs for biological modeling, maintaining data bases like those for protein and DNA sequences, providing long-term support for collections of cloned genes and useful vectors or collections of monoclonal antibodies.

3. continue support of mammalian models and the search for additional mammalian models.

4. consider supporting development of new model systems for specific research areas.

5. consider developing a clearing house encouraging the use of nonmammalian systems for testing the effects of exposure to chemicals of interest to environmental toxicologists.

6. consider encouraging interest in nonmammalian systems through fellowships, symposia, and direct support of model development.

7. leave the selection of the best system or organism for proposed research to the individual investigator.

8. investigate the matrix of biological knowledge concept as a potential tool for biomedical research.

This scholarly document provides a foundation for the NIH's consideration of the recommendations it contains and clearly demonstrates the value and role of diverse model systems in medical research.

Activities of the Section

The Biological Models and Materials Resources Section has begun activities responsive to recommendations 2, 4, 6 and 8 in the Academy report--recommendations similar to requirements to the Research Extension Act, P.L. 99-158 (Section 4). Further, the Section is serving as a home for several important research resources.

The Section is fully supporting two important research resources, the American Type Culture Collection and the Cell Culture Center at the Massachusetts Institute of Technology, and is sharing support for three additional resources whose primary support is with another NIH Institute (i.e., the Repository of Human DNA Probes and Libraries, the National Diabetes Research Interchange, and the Caenorhabditis elegans Genetics Center).

Each of these resources provides models or materials to the research community which meet the original objectives and reasons for implementation of the Biological Models and Materials Resources Section.

Description of the Resources the Section Supports

1. The American Type Culture Collection (ATCC)

The ATCC serves as a national repository and distribution center for a diverse collection of animal viruses, bacteria, bacteriophages, cell lines, fungi, plant viruses, protists, hybridomas, plant tissues, recombinant DNA vectors and oncogenes. This Section administers a contract which supports the curatorial functions of this unique resource. This resource responds to requests for over 70,000 cultures and cell lines each year and is the primary source of microbiological standards for the scientific community. These organisms are important to the full spectrum of NIH-supported biomedical research from basic to clinical investigations.

2. The Cell Culture Center at the Massachusetts Institute of Technology (MIT)

The Cell Culture Center at MIT provides a customized service for research investigators needing extremely large quantities of cells in culture or their products in their research. The primary mission of the Center is to produce cells and cell products on a large scale to allow scientists throughout the United States to conduct novel and important experiments in basic biology that could not be accomplished with the materials and resources available in their own laboratories. A wide range of investigators studying cellular and molecular biology use the Center's services. The Center is supported through a cooperative agreement and has provided services to investigators throughout the Nation.

3. The Repository of Human DNA Probes and Libraries

The use of DNA probes has revolutionized the conduct of genetics research, diagnosis, and therapy. The Biological Models and Materials Resources Section is sharing support, with the National Institute of Child Health and Human Development, of a Repository of Human DNA Probes and Libraries. The contract to support this project was awarded to the American Type Culture Collection in September of 1985. The Repository will establish a collection of cloned human genes, DNA probes, and human chromosome-specific libraries and serve as a major international resource center for the distribution of the rapidly proliferating human DNA clones and libraries. Probes and cloned genes are being actively sought from the genetics and molecular biology research communities. The human chromosome-specific libraries are being made available from a collaborative project supported by the Department of Energy at the Los Alamos and Lawrence Livermore National Laboratories. The Repository will also make available, online, a computerized data base on the repository holdings as well as background information on the probes and chromosome-specific libraries for use by interested researchers. The Repository is expected to assume a vital role in supporting research in genetics and molecular biology as well as in supporting the use of recombinant DNA gene mapping technology in mapping the human genome.

4. The National Diabetes Research Interchange (NDRI)

Research investigators who wish to corroborate findings established in animal models by conducting additional studies in human tissues have often experienced difficulty in obtaining these tissues. The NDRI was established in 1980 to meet this need. While the NDRI's primary funding comes from private foundations and the National Institute of Diabetes and Digestive and Kidney Diseases, the National Heart, Lung, and Blood Institute and the Division of Research Resources are also contributing to the support of this unique resource. Though originally specializing in collecting, preserving and distributing diabetic tissues for researchers, the NDRI has expanded its activities and is now supplying a wide range of healthy and diseased tissues and organs to an ever-expanding research community. Over 100 human tissue types have been supplied to investigators studying such diseases as diabetes, retinitis pigmentosa, cardiovascular disease, cystic fibrosis, and glaucoma.

5. The Caenorhabditis elegans Genetics Center

The Caenorhabditis elegans Genetics Center is a repository and distribution center for a small multicellular invertebrate, a species of round worm, that is finding increased utility as a model system for a wide array of fundamental studies in the biological sciences. Developed initially as a model for studies of the genetic control of development, it is showing increased utility in fundamental studies of neurobiology, endocrinology, and aging.

While the Center's primary support is provided through a contract from the National Institute on Aging, both the Biological Models and Materials Resources Section and the National Institute of General Medical Sciences are providing partial support for this resource.

Activities the Biological Models and Materials Resources Section is Planning

Two workshops were held in 1985 to examine the Academy's recommendation that NIH consider investigating a concept which developed in the evaluation study, referred to as the "matrix of biological knowledge," as a tool, and a potential resource, for biomedical research. The concept, theoretical in nature, and involving an interplay between experimental biology, information management and developments in the field of artificial intelligence, was viewed as a timely and necessary undertaking whose accomplishment, however, was seen as potentially massive and long-term. Experts from various fields and staff from the National Library of Medicine and other Federal agencies participated in these workshops. The conferees were enthusiastic about the concept and recommended that the Biological Models and Materials Resources Section, in conjunction with other interested agencies, organize a more extensive workshop of several weeks' duration, to more fully examine the potential benefits inherent in the concept. The goal would be to clearly define the practicality of attempting to generate what amounts to a national biomedical data storage, data retrieval and data management system. Such a system would access a range of different data banks. This concept would not generate one gigantic data bank but rather a system for accessing individual data banks and allowing communication between them. At its best this approach is seen as effecting a long-term saving of both money and experimental materials, providing maximal utilization of the information already purchased. It is seen as an attempt to remove existing constraints on an investigator's ability to access all information relevant to his or her studies. It would enhance choices of models suited to their investigations and increase the likelihood that both the unique and general characteristics of the biological phenomena under investigation become apparent.

The Section is proceeding with plans for the extended workshops that were recommended, and is seeking input and support from the other Institutes and Federal agencies which have expressed an interest in this concept's potential as a useful research resource.

This concludes my prepared statement. I will be pleased to answer any questions the Subcommittee may have.

Mr. WALGREN. All right.

Well, let's turn then to Dr. Rall.

Dr. RALL. Thank you, Mr. Chairman. I am very pleased to be here. I am David Rall the Director of the National Institute of Environmental Health Sciences, NIEHS; and of the National Toxicology Program, NTP. We have worked diligently and made considerable progress in the development and validation of improved toxicity research and testing methods.

I shall focus primarily on the work of NIEHS and NTP, as requested; and I should point out that the animals used are almost exclusively rats and mice in our studies. And I wish to state I know nothing about dairy cows.

The Toxicology Research and Testing Program and NIEHS, comprises the central core of the National Toxicology Program. NTP, now in its eighth year, is a cooperative effort of NIEHS, the Food and Drug Administration's National Center for Toxicological Research and the Center for Disease Control's National Institute of Occupational Safety and Health, all of these within the Department of Health and Human Services.

The purpose of NTP is to strengthen the science base in toxicology and to coordinate research and toxicology studies on potentially toxic compounds. This information is used by regulatory and research agencies as well as by other organizations concerned with the public's health.

The information is peer reviewed and is publicly available either in technical reports, which are announced in the Federal Register, or in the NTP's annual plan and report, and in the NTP's survey of toxicological studies in progress within HHS, the Department of Energy and the Environmental Protection Agency, which covers the very large bulk of the nonclassified toxicological research within the Federal Government.

Until the development of modern toxicology, the association between chemical exposure and health effects became apparent for many serious effects only after exposure took place, often at cost of great human suffering and deaths. With the evolution of toxicology we now have laboratory approaches to identify some of these hazards and to understand their effects in laboratory and laboratory animals and ultimately in human populations.

Essential to this premise is the knowledge derived from basic research, that biological processes of molecular, cellular, tissue, and organ functions that control life are strikingly similar from one mammalian species to another. Processes such as sodium and potassium transport, iron regulation, energy metabolism, DNA replication vary little in the aggregate as one moves along the phylogenetic ladder. The whole study of the genetic events has a strong thread of similarities from the smallest virus to largest mammal. The classic work on the transmission of neural impluses in the squid axon is directly relevant to human neuromuscular behavior. Advances in our understanding of neurological disease based on laboratory work has only been achieved through the use of whole animal models. The immunological effects of dioxin, TCDD, were first observed in mice in the mid-1970's, and thus predicted the results of immunological studies reported a week or two ago in Quail Run, the Missouri community exposed to TCDD-contaminated dirt.

It is in chemical carcinogenesis where the benefits of the use of laboratory animals have been most apparent. From experimental studies we have learned that if a chemical is carcinogenic in appropriate laboratory animal systems, it is likely to be carcinogenic in man. And in fact, we note that six of the human carcinogens were first shown to be carcinogenic in laboratory animals. The information generated by our toxicological studies is often used as the basis for regulatory action. And this requires a highly rigorous standard of proof. It is imperative therefore that the quality and conduct of our comprehensive toxicological characterizations and methods development meet the highest scientific standards.

Our work investigating new methods is long, arduous, and expensive, but one in which we have made notable progress. There are number of ways in which NIEHS and NTP contributed both directly and indirectly to the use of fewer laboratory animals.

Traditionally approaches using whole animals require that groups of animals be autopsied at varying times after being exposed to the chemical in order to follow the progression or regression of a toxic lesion. This means that extra animals are required at each point in time.

Recent advances in complicated techniques called magnetic resonance and magnetic resonance imaging make it possible now to carry out noninvasive studies on intact, anaesthetized animals. We can observe in intact animals the development of many lesions, including tumors and we can, if necessary, observe their regression all in the anaesthetized but otherwise perfectly normal animal.

Evaluation of the use of these various instruments in experimental animals for this purpose is still in the developmental phase. But I think, I am quite optimistic that this will create a revolution in the toxicological research and testing efforts.

There are other studies done at NIEHS that have directly or indirectly affected the number of animals used in research and testing. We now evaluate more parameters or end-points on a given group of animals reducing the need, again, for extra animals.

In our prechronic studies we also evaluate studies on reproduction, immune function, and genetic toxicology; historically, separate studies, and therefore separate groups of animals had been required.

Starting in the early 1970's NIEHS, and later NIEHS and NTP, began the development of alternative test systems that would improve our ability to identify and understand environmental health hazards. Research funds were awarded early on to evaluate if flowering weeds and other plants that might identify mutagenic air pollutants.

Generations of fruit flies were studied as possible alerting systems for human health hazards. Cells from humans were cultivated in the laboratory for use in cancer studies.

NIEHS and NTP scientists are involved currently in the development and refinement of a number of assay systems, that among other thing, result in the use of fewer animals.

I have included in my testimony a list of these systems, and I would be delighted to furnish further information.

Having shared my optimism over the promise that these approaches offer, I must also caution that short-term tests can provide solid information, they must not be overly interpreted. Before they can be used with confidence we need to confirm that they are providing the information that we expect them to provide. They can be very useful as rapid, inexpensive means of capturing data on specific potential toxicity, but their value as predictive tools is still evaluated.

This is a lengthy and expensive process. NIEHS and NTP have devoted substantial effort over the last few years to laying a firm technical and scientific foundation for an evaluation of this issue.

We have devoted approximately $70 million to this effort over the last 5 years, and we expect this will continue into the future. There are well over 100 assays that have been proposed as substitutes for predicting or studying toxicological effects.

The utility of any system is first dependent upon its reproducibility within and between laboratories. Therefore, in our evaluation of such assays we have adopted the principles derived from clinical research of double blind testing.

Drawing on our own extensive data base—and there is no other like it in the world—NIEHS and NTP has established a process for systematically evaluating the correlation between results in short-term mutagenicity screens and results of chronic carcinogenicity studies.

This process is ongoing at the moment because of the very large number of data points, it will be the summer or fall before we have definitive information. I will insure that the committee is made aware of these results as soon as they are available.

In order to stimulate concern within the scientific community at large for the development of alternatives to the use of animals in basic and applied research NIEHS last fall issued request for grant applications directed toward the development, validation and use of nonmammalian methods for the study of biological effects and toxicity of environmental agents.

As a major of the interest within the biomedical community we have already received almost 40 applications, and we expect that we will begin to award some of these in early fiscal 1987.

I might add parenthetically that we receive a number of investigator initiated applications under the Small Business Innovative Research Act, the SBAR, which deal with innovative new uses for toxicity testing. I don't have those numbers at hand, but it has been successful in that area, also.

Mr. **Walgren**. If I could ask, how does that set-aside work here? I understand we are supposed to allocate 2 percent or something like that to small business. Is that on an NIH total, and so if there is a concentration in this area that would apply to your total; or do you do 2 percent?

Dr. **Rall**. It is basically 2 percent on an institute basis. We indicated the areas we are interested in, and alternatives to animal testing is one of those areas. And we have, as I said, a number of applications.

I will be brief; I think you can read my statement.

The NTP and NIEHS have been at the forefront in efforts to develop alternative methods for identifying, explaining the toxicological effects of chemicals on biological systems. We are doing this in a number of ways, improvements in study design, investigation of many new short-term methods and so forth.

Again, let me warn that a long, important, massive evaluation is required. This long, complex, and more costly process, will provide a more exact indication of the predictive value of these short-term toxicological studies.

Thank you, I would be delighted to answer any questions.

[The prepared statement of Dr. Rall follows:]

Statement of

David P. Rall, M.D., Ph.D.
Director, National Institute of Environmental Health Sciences
Director, National Toxicology Program
National Institutes of Health
U.S. Department of Health and Human Services

Before the
Subcommittee on Science, Research and Technology
of the
Committee on Science and Technology
U.S. House of Representatives

May 6, 1986

Introduction

I am David P. Rall, Director of the National Institute of Environmental Health Sciences (NIEHS) and Director of the National Toxicology Program (NTP). I am delighted to have the opportunity to describe our recent progress in development and validation of improved toxicity research and testing methods. Much of our work to develop alternative toxicological methods results in a reduction in the numbers of animals used. In my remarks I will focus on the work of the NTP, as the Committee requested. It is important to note at the outset, that in NTP's toxicological studies the animals used are almost exclusively rats and mice.

The Toxicology Research and Testing Program at NIEHS comprises the central core of the National Toxicology Program. NTP, now in its eighth year, is a cooperative effort of NIEHS, the Food and Drug Administrations's National Center for Toxicological Research and the Centers for Disease Control's National Institute for Occupational Safety and Health, all within the Department of Health and Human Services. NTP's purpose is to strengthen the science base in toxicology and to coordinate research and toxicology studies on potentially toxic chemicals. This information is used by Federal regulatory and research agencies as well as by other organizations concerned with the public's health.

Our society places extraordinary value on protection of the public's health. In providing that protection we must use the knowledge and tools that we have available to identify and then minimize risks to human health. Ideally, this should be done before people become sick or die. Currently, animals are the best surrogate we have for humans and, for those laboratory studies that require whole animals, we must use a statistically adequate number to allow a reasonable power of detection of effects from chemical exposures. However, the conduct of this work must be guided by strict ethical considerations, including the highest standards for humane treatment of animals utilized in achieving this societal goal.

CHEMICAL TOXICOLOGY

Until the development of modern toxicology, the association between chemical exposure and health effects became apparent usually only after the exposure took place, often at a cost of great human suffering and death. With the evolution of toxicology, we now have laboratory approaches to identify some of these hazards and to understand their effects in laboratory animals and ultimately in humans.

Theoretical consideration and experience indicate that it is possible to identify the effects of chemicals in laboratory animals to which humans are or will be exposed, and to use these results to predict in general terms what is likely to occur in the human population. Essential to this premise is the knowledge, derived from considerable basic research, that biological processes of molecular, cellular, tissue, and organ functions that control life are strikingly similar from one mammalian species to another. Processes such as sodium and potassium transport and ion regulation, energy metabolism, and DNA replication vary little in the aggregate as one moves along the phylogenetic ladder. The whole study of genetic events has a thread of similarities from the smallest virus to the largest mammal. The classic work on the transmission of neural impulses in the squid axon is directly relevant to humans. Advances in our understanding of neurological disease based on laboratory work has only been achieved through the use of whole animal models. At present it is impossible to mimic the nervous system in in vitro* models. The immunological effects of dioxin (TCDD) were first observed in mice in the mid-1970s thus predicting the results of immunological studies of the residents of Quail Run, the Missouri community exposed to TCDD-contaminated dirt.

It is in chemical carcinogenesis+ where the enormous benefits of the use of laboratory animals have been most apparent. From experimental studies we have learned that if a chemical is carcinogenic in appropriate laboratory animal test systems, it is likely to be carcinogenic in humans. It is important to note that 4-aminobiphenyl, diethylstilbestrol (DES), mustard gas, vinyl chloride, aflatoxins, bis(chloromethyl)ether (BCME), and melphalan were shown to be carcinogenic in laboratory animals prior to evidence that they were carcinogenic in humans.

One of the major developments in chemical carcinogenesis has been the demonstration that a family of genes that reside in normal cells can be activated by chemicals to become oncogenes, substances whose protein products contribute to the process of malignancy. It is inconceivable that we could have achieved this understanding of the role of oncogenes without extensive use of laboratory animals. We now know that certain chemicals initiate the cancer process while others promote it. Such an understanding should eventually allow us to better classify carcinogens based on their mechanism of action, thus improving the ability to assess health risks and take appropriate regulatory action.

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*in vitro: Study of biological effects or processes in other than the whole animal.

+chemical carcinogenesis: Study of a chemical's ability to produce or incite cancer.

Positive results in long-term carcinogenesis animal studies by NTP have had significant regulatory consequences. A few examples include 1,3-butadiene (a chemical used in the production of rubber products) for which the Environmental Protection Agency (EPA) initiated a review of the chemical and then referred it to the Occupational Safety and Health Administration (OSHA) for possible regulatory action. OSHA is soliciting comments regarding the health risk of exposure. As another example, methylene chloride carcinogenesis results were utilized by EPA in deciding to initiate a priority review. Recently the Food and Drug Administration (FDA) published a rule to ban methylene chloride's use in cosmetic products. Also, the Consumer Product Safety Commission (CPSC) is considering a number of actions for decreasing exposure to methylene chloride in paint strippers and spray paints. On a third chemical, ethylene dibromide, EPA has made a decision to eliminate certain uses as a pesticide.

Through the use of animals, toxicity studies by the NTP have also provided the public with some degree of confidence that certain chemicals, drugs and vitamins such as xylenes (a constituent of gasoline), ephedrine sulfate (a sympathominetic -- a central nervous system stimulant used as a broncho dilator) and vitamin C do not cause toxic or carcinogenic effects based on studies in rodents exposed to relatively high amounts of these compounds.

Because the information generated by our studies is often used as the basis for regulatory action, which requires a highly rigorous standard of proof, it is imperative that the quality and conduct of our comprehensive toxicological characterization studies and methods development meet the highest scientific standards. Our work investigating new methods is a long, arduous, and expensive undertaking, but one in which we have made notable progress. The Committee should understand that until we can guarantee the public that these new methods are reliable, reproducible and measuring what they are intended to measure, it will be necessary to use a number of animals for a period of time.

ALTERNATIVES TO THE USE OF ANIMALS

There are a number of ways in which NIEHS and NTP contribute both directly and indirectly to the use of fewer laboratory animals. A good example of this is NTP's study of chemical disposition. This approach uses a limited number of animals to determine how a chemical is absorbed, where it migrates in the body, how long it is retained, in what form, and how and where it is excreted. This type of information can result in better scientifically designed studies that require fewer animals overall.

Another example of large savings in numbers of laboratory animals and dollars is the NTP's benzidine dye initiative. This group of dyes includes some 82 discrete chemicals that are available in the United States. Because of the high cost and time requirements for long-term studies in rats and mice, the aim of this effort has been to develop an integrated body of scientific knowledge concerning the pharmacokinetics*, genetic toxicology, and systemic toxicity and carcinogenicity of these chemicals as a class. Through the careful selection of chemicals it should be possible to establish basic principles that can be applied to the entire class of compounds. Thus, it will not be necessary to conduct long-term studies on every chemical in this class. From such studies of the relationship between a chemical's structure and its activity in biological systems we can answer some additional questions rather than put related chemicals into animal studies.

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*pharmacokinetics: Study of the rate of absorption, distribution, metabolism, and excretion of substances from the body.

Traditional approaches using whole animals typically require that the animals be autopsied at varying times after being exposed to the chemical in order to follow the progression or regression of a toxic lesion. This means that a sufficient number of animals is required at each time point to ensure statistical validity. Recent advances in magnetic resonance (MR) and magnetic resonance imaging (MRI) instrumentation may make it possible to carry out non-invasive studies on intact, anaesthetized animals. Thus, after exposure to a chemical the effect in the biological system can be monitored continuously in the same animal over a period of hours, days or longer if necessary, thereby decreasing the need for additional animals. The development of tumors, as well as their regression, can also be followed in this way. This ability to carry out long-term studies on individual animals over time reduces the need for comparisons among different animals and thus can lead to a reduced need for animals. In addition to providing a non-invasive method to study chemical effects in whole animals, this methodology can be used in studies of tissue culture. In such studies the biotransformation of the chemicals can be continuously followed and the effects on various cellular parameters monitored.

Evaluation of the use of the MR and MRI instrumentation in experimental animals for this purpose is still in the development phase. Based on our study of these techniques so far I am quite optimistic that they will be extensively integrated into our toxicology testing efforts in time.

There are several other studies that have been done at NIEHS that have either directly or indirectly affected the numbers of animals used in our research and testing. As a result of more effective dose targeting, we have begun to reduce the number of dose groups in some of our pre-chronic studies. Also, we are now able to evaluate more parameters on a given group of animals, reducing the need for as many animals. For example, we use our pre-chronic studies to also evaluate effects on reproduction, immune function and genetic toxicity. Historically, separate studies, and therefore separate groups of animals, were required to evaluate each of these parameters. In addition, this has provided us an even better indication of whether or not a long-term study is needed and if so an improved ability to design the study to get at the information we want. In other words, we are using the same or fewer numbers of animals to get more information and to design studies better in order to avoid the need for repetition.

DEVELOPMENT OF ALTERNATIVE TEST METHODS

Starting in the early '70s NIEHS, and then later with the creation of NTP in 1978, committed to the development of alternative test systems that would improve the ability to identify and understand environmental health hazards. Research funds were awarded early on, for example, to evaluate weeds and other plants that might identify air pollutants. Generations of fruit flies were studied as a possible alerting system for hazards to human fetuses. Cells from humans were cultivated in the laboratory for use in cancer studies. Microbial cells and tissues were being utilized by many scientists in studying injury to specific organs.

Currently, one of our major objectives in improving test methods is the development of short-term tests that provide an indication of the need for longer-term chronic testing. Such methods enhance the ability to set priorities for testing of chemicals and subsequently aid in the design and interpretation of long-term animal studies. NTP has in place a testing rationale that involves the execution and evaluation of certain short-term test results prior to making a decision to carry out a two-year animal study.

In their efforts to understand more clearly the mechanisms of chemically induced damage at the cellular and molecular level, both NIEHS and NTP scientists are involved in the development and refinement of assay systems that may, among others things, result in the use of fewer animals. To give you a sense of the scope and diversity of this effort I would like to list for the Committee some of the approaches being examined:

. Cell culture system to study a multistep model of carcinogenesis
. In vitro screening system for teratogens*
. Whole mouse embryo culture for study of teratogenesis
. Use of isolated brain cell components of the rat to measure effects of drug release on hormones of reproductive system

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*teratogen: Factor that causes production of defect in the developing embryo.

- Cell culture for study of toxic effects in the kidney
- Human tissue culture technique to compare human with rodent metabolism
- Continued development work on the Salmonella (Ames) mutagenesis[**] assay
- Aneuploidy[*] test system to monitor chemically-induced aneuploidy
- Human cell assay for genetic toxicity
- Non-invasive test for neurological deficits in the whole animal
- Drosophila mutagenesis test and teratogenesis screen
- Algae as model of mammalian metal metabolism

EVALUATION OF ALTERNATIVE TEST METHODS

Having shared my optimism over the promise that these approaches offer, I must also caution the Committee that although short-term tests can provide solid information, they must not be overly interpreted. That is, before they can be used with confidence we need to confirm that they are providing the information that we expect them to provide. They can be very useful as a rapid, inexpensive means of capturing data on specific potential toxicity, but their value as predictive tools is still being evaluated. This is a lengthy and expensive process. NIEHS and NTP have devoted substantial effort over the last several years to laying a firm technical and scientific foundation for an objective evaluation of this issue.

There are well over 100 various assays that have been proposed to be potential substitutes for predicting or studying toxicological effects in the whole animal and humans. However, the utility of any system is first dependent on its reproducibility within and among laboratories. Therefore, in our evaluation of such assays we have adopted the principles derived from clinical research of double blind testing where the chemicals to be evaluated are tested under code and the results evaluated for positive or negative response by predetermined criteria before the code on the chemical is broken. We believe this approach is essential to minimize investigator bias and the influence of preconceptions on evaluation of results.

[**] mutagenesis: Study of changes in genetic material.

[*] aneuploidy: An abnormal number of chromosomes.

Drawing on its own extensive data base -- there is no other like it in the world -- NTP has established a process for systemmatically evaluating the correlation between results in short-term mutagenicity screens and results of chronic carcinogenicity studies. This evaluation process is difficult and time consuming since it must be a multifactorial process that includes consideration of type and magnitude of effect in the whole animal and in the short-term system, as well as relation to chemical class and structure.

We are very hopeful that out of this sort of objective and systematic evaluation effort will emerge a clearer picture of the uses and limitations of short-term test systems as predictive tools for the effects of chemicals in the whole animal. We also know that not all of our questions will be answered by this undertaking and that additional studies will be needed to achieve our ultimate goals. As our initial evaluation is completed, we will make our results public. I will ensure that the Committee is made aware of them as soon as they are available.

OTHER EFFORTS

In order to further stimulate concern within the scientific research community at large for the development of alternatives to the use of animals in basic and applied research NIEHS has issued a request for grant applications directed toward development, validation and use of non-mammalian methods that can be employed to study the biological effects of environmental agents. Almost forty applications have been received in response to this announcement. We expect that approved applications will be awarded in early FY 1987.

SUMMARY AND CONCLUSIONS

The field of toxicology has matured to the stage where results from laboratory animal studies can provide reasonably good indication of a chemical's health effects when that chemical is investigated under the appropriate conditions. Our confidence stems from experience indicating correlation from one mammalian species to another and from laboratory animals to human populations. As the examples I mentioned demonstrate, the information gained from experimental animal studies can have significant public health and regulatory implications.

Scientists are continuously seeking improved, more precise ways to develop this information. This endeavor is central to the process of scientific investigation and is complemented by the humane desire to decrease the numbers of animals used for research and testing. The natural accumulation of knowledge will stimulate development of alternative approaches, some of which may lead to the use of fewer laboratory animals. There are however, areas of study for which no reliable alternative to the intact animal is now possible. Faced with the need to demonstrate that the use of a new chemical will not pose an unacceptable risk to human health, it is crucial that we understand the effects (if any) of the new chemical on each organ system and on the vital functions of the animal.

The National Toxicology Program has been at the forefront in efforts to develop alternative methods for identifying and explaining the toxicological effects of chemicals in biological systems. We are doing this in a number of ways, ranging from improvements in study design and use of pre-chronic study data, to investigation of a number of short-term test methods. Before we can be thoroughly confident that these short-term tests are reproducible and reliable, measuring what they are intended to measure, extensive evaluation is required. This is a long, complex and costly process, but one that will provide a more exact indication of the predictive value of short-term toxicological studies.

This concludes my prepared remarks. I would be happy to answer any questions that you might have.

CURRICULUM VITAE

David Platt Rall

Birthplace and Date:	Aurora, Illinois; August 3, 1926
Marital Status:	Married; two children
Education:	Public Schools, Naperville, Illinois B.S., North Central College, Naperville (1946) M.S. (Pharmacology), Northwestern University (1948) Ph.D. (Pharmacology), Northwestern University (1951) M.D., Northwestern University School of Medicine (1951)
Professional Training:	Internship; Second (Cornell) Medical Division, Bellevue Hospital, 1952-53
Experience:	Director, National Institute of Environmental Health Sciences, NIH, 1971-present Director, National Toxicology Program, 1978-present Assistant Surgeon General, USPHS, 1971-present Associate Scientific Director for Experimental Therapeutics, National Cancer Institute, 1966-71 Medical Director, USPHS, 1963-71 Chief, Laboratory of Chemical Pharmacology, National Cancer Institute, 1963-69 Senior Surgeon, USPHS, 1959-69 Head, Clinical Pharmacology and Experimental Therapeutics Service, General Medicine Branch, National Cancer Institute, 1958-63 Scientist, Clinical Pharmacology and Experimental Therapeutics Service, General Medicine Branch, National Cancer Institute, 1955-58 Surgeon, USPHS, 1955-59 Scientist, Laboratory of Chemical Pharmacology, National Cancer Institute, 1953-55 Research Associate in Pharmacology, Northwestern University, 1950 Assistant in Pharmacology, Northwestern University, 1949-50 Baxter Fellow in Pharmacology, Northwestern University, 1947-49

David Platt Rall

Association Memberships:

American Association for the Advancement of Science
American Association for Cancer Research
American Public Health Association
American Society for Clinical Investigation
American Society for Pharmacology and Experimental Therapeutics
Federation of American Societies for Experimental Biology
Society for Occupational and Environmental Health
Society for Risk Analysis
Society of Toxicology

Honors and Other Special Scientific Recognition:

Member, US Delegation to the US-Japan Cooperative Science Committee, 1984-present
Arnold J. Lehman Award, Society of Toxicology, March 1983
Editorial Board, *Regulatory Toxicology and Pharmacology*, 1981-present
Editorial Board, *Risk Analysis*, 1981-present
Head, DHHS Delegation, US/Japan Non-Energy R&D Cooperative Agreement Joint Committee Meeting, Tokyo, Japan, September 1981
Chairman, WHO Programme Advisory Committee, 1980-81
Member, WHO Programme Advisory Committee, 1980-present
Editorial Board, *American Journal of Industrial Medicine*, 1980-present
Member, Institute of Medicine, National Academy of Sciences, 1979-present
Nellie Fox Lecturer, Northwestern University Medical School, April 1978
Chairman, Committee on Health and Environmental Effects of Increased Coal Utilization, 1977-78
Harrington Lecturer, University of Buffalo, April 1976
Member, NAS Safe Drinking Water Committee, 1975-1977
Member, DHEW Secretary's Review Panel on New Drug Regulation, 1975-76
Associate Editor, *Journal of Toxicology and Environmental Health*, 1974-78
Editorial Board, *Archives of Environmental Health*, 1973-76
Chairman, DHHS Committee to Coordinate Environmental and Related Programs, 1973-1985

David Platt Rall

Chairman, Subcommittee on Environmental and Occupational Health, Joint Working Group on Health Cooperation, US/Egypt Cooperative Agreement, 1972-present
US Coordinator, Environmental Health Program, US-USSR Health Exchange Agreement, 1972-present
US Coordinator, Biological and Genetic Consequences Project, US-USSR Environmental Protection Agreement, 1972-present
US Coordinator, US-UK Cooperative Program in Environmental Health Sciences, 1972
Adjunct Professor, University of North Carolina, Chapel Hill, 1972-present
Chairman, OST-CEQ Ad Hoc Committee on Environmental Health Research, 1971-72
Associate Editor, *Cancer Research*, 1970-74
Chairman, DHEW Departmental Committee on Drug Research and Regulations, 1970
Editorial Board, *Pharmacological Reviews*, 1969-72
Chairman, National Institute of General Medical Science Pharmacology-Toxicology Review Committee, 1967-69
Member, National Institutes of Health Chemotherapy Study Section, 1965-67
Editorial Board, *Proceedings of the Society for Experimental Biology and Medicine*, 1962-65
Lecturer in Physiology, Medical School, George Washington University, 1958-62
PHS Meritorious Service Medal, 1966
DHEW Distinguished Service Medal, 1975

Research Interests:

Dr. Rall has authored and co-authored approximately 150 published papers relating to comparative pharmacology, cancer chemotherapy, blood-brain barrier, blood CSF barrier, pesticide toxicology, and drug research and regulation.

Mr. **Walgren**. Thank you, Dr. Rall, we appreciate that.

Dr. Guest?

Dr. **Guest**. My name is Gerald Guest, I am with the Center for Veterinary Medicine, at the Food and Drug Administration. I do have a very brief statement, if I may.

The FDA, like other components of the Department of the Health and Human Services, is actively pursuing a number of initiatives to insure the most humane treatment possible for test animals and to reduce or eliminate entirely requirements for such tests as the Draize or the LD-50.

In their testimony this morning Drs. Rall and Willett of the National Institutes of Health presented, I think, an excellent overview of the Department's efforts to minimize the use of animals for testing. I think they correctly emphasized, however, that since animals are the best surrogates for humans, there will be a continuing, albeit, I hope, decreasing need for the use of animals in research in order to minimize risk to human health. I think the need for some testing is reflected in the principal law that is administered by FDA, that is the Federal Food, Drug and Cosmetic Act.

The act imposes on manufacturers the burden of demonstrating that their products meet the safety requirements of the law. Apart from the work done at our National Center for Toxicological Research in Arkansas, FDA conducts relatively little toxicology testing of its own.

Instead, we recommend the type and extent of testing we believe necessary to determine safety, and then review the data that are submitted by drug and food manufacturers to determine whether they meet these requirements.

Thus, we require that all human and animal drugs and food additives undergo careful testing in animals to assess their potential toxicity to man; to determine whether they have any teratogenic potential; and to determine carcinogenicity when there is a likelihood of chronic exposure.

Animal studies of human drugs are of particular importance in determining whether new products can safely be tested in humans and to assess their potential therapeutic effect. Obviously, I believe it would be neither legal or ethical to begin human trials until its acute toxicity and other harmful potential effects have been carefully tested in animals.

Chemicals used as drugs to treat animals or feed additive products require toxicity testing for several reasons. One reason is the requirement to assure safe use in that target animal, whether it be a horse, a cat, or a dog. And another is the necessity to assure that chemicals used in food-producing animals are safe if they become a component of human food as a residue.

Despite the need for animal testing to some degree, FDA has already undertaken or plans to undertake efforts to reduce the use of animals in research and testing, and to avoid unnecessary testing methods.

On November 9, 1983, FDA sponsored an acute studies workshop attended by approximately 150 persons from Government, industry, and public interest groups. The purpose of that workshop was to discuss the scientific rationale, requirements and uses for acute toxicity studies, including lethality, to clarify the regulatory requirements for acute toxicity data, and whether there was any longer a need for a statistically exact LD-50 value, or the dose which kills 50 percent of a group of a laboratory animals under study.

The following consensus points emerged from that particular workshop. There was general agreement among Government and industry representatives that the LD-50 test is often credited with greater quantitative and scientific accuracy than it merits, and that there are are other determinants of acute toxicity such as site and mechanism of action, which are certainly more desirable in expanding the scope of our knowledge in the toxicity area.

The requirements for the LD-50 tests among Government agencies and industry is much less than that perceived by the general public. For example, FDS does not require the use of the LD-50 test to assess the safety of the products it regulates.

Point No. 3, industry and Government agencies support the development and validation of alternative methods, those using as few animals as possible and those that use no animals.

Point No. 4 in the consensus was that the U.S. Government agencies are cooperating on animal welfare issues with other countries through organizations such as the Organization for Economic Cooperation and Development.

As a result of this workshop, FDA established an agency-wide steering committee on animal welfare issues to review its guidelines on the use of animals and to recommend changes where needed through the Commissioner of Food and Drugs. On August 15, 1984, the steering committee issued its final report which called for, among other recommendations, a greater coordination between FDA centers in the use and development of in vitro alternatives to animal testing; instituting more uniform agencywide practices for the care and handling of animals; and establishing a permanent FDA animal welfare committee.

These recommendations were adopted and at present we have an active animal welfare committee in place. We would like to submit a copy of this steering committee's report for the record, Mr. Chairman.

Mr. WALGREN. Without objection, we would be happy to have it.

[The report follows:]

FINAL REPORT TO THE COMMISSIONER

FOOD AND DRUG ADMINISTRATION

AGENCY STEERING COMMITTEE ON ANIMAL WELFARE ISSUES

August 15, 1984

U.S. Department of Health and Human Services
Public Health Service
Food and Drug Administration
Rockville, Maryland 20857

Executive Summary

The Food and Drug Administration (FDA) Steering Committee on Animal Welfare Issues was formed in January 1984. Representatives from each Center and the Office of the Commissioner addressed the five issues with which they were charged by gathering information on Agency-wide procedures, practices and requirements related to each issue. As a result of studying and analyzing much information provided by staff members from each Center, the Committee has reached the following conclusions.

A. General Observations

1. For many years the FDA has demonstrated concern for the proper care and treatment of animals in its research and testing programs.

2. Representatives from FDA have been working for several years with organized groups at the National Institutes of Health and the U. S. Department of Agriculture to assure humane treatment of animals.

3. There have been continuing efforts, again for a number of years, to review testing requirements and to minimize the use of animals needed to meet those requirements.

4. While much progress is being made on the development of certain alternative test procedures, animals will remain essential to medical and health research, safety determinations, and risk assessment for the foreseeable future.

B. Specific Observations

1. FDA practices and procedures are designed to obtain the maximum amount of data from the minimum number of animals. This is accomplished in the Centers by a continual review of requirements and new techniques, protocol reviews for research projects, general oversight in various ways such as by having written requirements, guidelines and procedures which specify what is needed for product approval.

2. FDA has no requirements for LD_{50} data obtained by using the classical, statistically precise test except for batch release toxicity tests of three antitumor antibiotics. The Agency is considering eliminating this requirement. The Committee found several references to

the LD_{50} test in older guidelines which are being rewritten to clarify the requirement for acute toxicity studies, including approximate lethal dose instead of LD_{50} tests.

3. There are many alternative tests being studied and developed throughout the Agency. Although most require more research for validation, some in vitro studies are useful as screening tools to provide guidance to determine if additional animal studies are required or can be omitted. Immunochemical and biochemical techniques are being substituted for animals to determine the potency and purity of some biological products. There is excellent potential for developing acceptable alternatives to the use of animals or their reduction in test numbers for some purposes.

4. Throughout the Agency there are practices and procedures for assuring humane care and treatment of animals. Two facilities are accredited by the American Association for Accreditation of Laboratory Animal Care, the highest formal accreditation, and the others have self-assessment procedures in place which meet or exceed Public Health Service standards. Centers continually review their procedures to reach and maintain the highest standards of humane care for animals.

5. FDA has a number of regular channels of communication to industry, consumers and the private sector in general. These are used for informing the FDA constituents of policies and procedures as well as for providing a means for these groups to communicate their questions and concerns to the Agency. Efforts to improve communication channels will continue.

Introduction

The FDA is responsible for protecting and promoting public health by assuring the safety of foods, drugs, cosmetics, biologicals, medical devices and radiological products. This responsibility covers products intended for human and animal use. To meet its responsibilities, the Agency conducts research and testing and requires premarketing safety data to establish safety-in-use for most products. Premarketing safety data are not required for radiological products, some medical devices or cosmetics except for colors used in the area of the eye.

In the recent past, some concern and confusion has been expressed by industry as well as by individuals and animal welfare organizations as to the exact nature of those requirements. A particular concern has been over requirements for acute toxicity data, especially the use of a statistically precise, traditional LD_{50} test. However, there is broader interest in the overall requirements for the use of animals in developing toxicological data and in the care and handling of animals in research and testing programs.

The FDA shares these same concerns and in an effort to address them has done two things. First, it sponsored a workshop on acute toxicity studies in November 1983. The workshop was open to the public and included participants from FDA and other government agencies as well as from industry. Its purpose was to discuss and clarify requirements for acute toxicity data. A report of the workshop was issued in February 1984. Second, the Agency announced at that workshop that it intended to form a committee to review the care and handling of animals throughout the Agency. A Steering Committee on Animal Welfare Issues was formed in January 1984 and charged with addressing the following issues:

1. Are FDA procedures so ordered as to obtain the maximum amount of useful scientific information while utilizing the fewest number of animals?

2. Do FDA procedures in any way indirectly stimulate the perpetuation of the LD_{50} test even though the Agency no longer directly requires the use of this test?

3. Is FDA making the maximum use of and encouraging the continued development of reliable _in vitro_ alternatives to _in vivo_ methodologies?

4. Are mechanisms in place to ensure continuing compliance with

the Animal Welfare Act and with the highest standards of animal care?

5. Is the historical usefulness of animal testing in human health protection, the primary mission of FDA, properly appreciated by our constituents?

All of the Centers and the Office of the Commissioner were represented on the Committee. The scientific backgrounds of the members included toxicology, pharmacology, veterinary medicine, microbiology and chemistry. Through its members, the Committee reviewed in-depth each Center's procedures and practices related to in-house research and research supported under contracts and grants. It also reviewed requirements imposed on industry for regulatory purposes. The staffs of the Centers were extremely cooperative in providing their representatives with data and information which the Committee studied and analyzed. This report presents their findings.

ISSUE #1 - Are FDA procedures so ordered as to obtain the maximum amount of useful scientific information while utilizing the fewest number of animals?

FINDINGS:

The FDA has established procedures which are intended to obtain the maximum amount of information from the minimum number of animals. For intramural research involving animals, scientists are required to have protocols reviewed and approved prior to initiating a project. Part of that review focuses on the appropriate use of animals and the design of the protocol to derive scientifically reliable data from the minimum number of animals. In addition to involving statisticians in protocol development and requiring review, the Agency makes continuing efforts to use *in vitro* and chemical methods to replace or minimize the use of animals in-house and in its requirements and recommendations to industry. This aspect is presented in greater detail in Issue #3.

Perhaps the most significant contribution to the minimization of the use of animals results from the issuance of guidelines for conducting tests required to produce data necessary for a toxicological characterization of products which FDA regulates. Because of the wide range of products agency-wide, an appreciable number of tests using a variety of animal species is required. A listing of these is shown in Tables 1-9. Despite the progress being made in the use of alternatives, animals are still necessary for assessing the safety of new products. By using valid, scientifically accepted testing guidelines in-house and as requirements for industry, the maximum amount of useful data is obtained using the fewest number of animals. Without guidelines which recommend the numbers and kinds of tests and animals, data generated from inappropriate numbers and kinds of tests might result in the conduct of more tests and use of more animals than is absolutely necessary. Depending on the product and proposed use, it may be adequate to determine only some and not all acute, subchronic and chronic effects and having guidelines helps in specifying requirements. Guidelines exist to define test protocols for evaluating safety of food and color additives, cosmetics, potency and safety of biologicals, human and veterinary drugs, and medical devices; and some exist as part of research protocols.

Several examples may illustrate the importance of written guidelines for minimizing the testing requirements and the use of animals. The Center for Food Safety and Applied Nutrition (CFSAN) has issued "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food." This document introduces a "concept of concern" which utilizes a tiered system for developing information for safety assessment. A procedure is outlined where, for the purposes of deciding the extent of toxicity testing needed to determine safety, a compound is placed into one of three levels of concern. Initially, information on structure-activity relationships and exposure data or estimates is used to assign a compound to a concern level. The document also lists the testing requirements for each concern level; the fewest number of tests being required for Concern Level I and the most extensive testing for Concern Level III. Test guidelines are included. The CFSAN specifically states that, "While this scheme does not preclude a petitioner from demonstrating safety by using other types of data elements, a submission using the Agency's scheme should normally provide sufficient scientific data to demonstrate safety."

The Center for Veterinary Medicine (CVM) is proposing a document, "General Principles for Evaluating the Safety of Compounds Used in Food-producing Animals" for widespread distribution and use. This contains guidelines for (1) metabolism studies and identification of residues for toxicological testing, (2) toxicological testing, (3) threshold assessment, (4) establishing a tolerance, (5) approval of methods of analysis for residues, and (6) establishing withdrawal periods. The CVM says in the introductory section that the sponsor is required to furnish information showing that residues in the edible products of treated animals are safe and the guidelines are intended to inform sponsors of the scientific information that provides an acceptable basis for such a determination. The "Principles" document specifically states, "Sponsors may rely upon the guidelines with the assurance that they describe procedures acceptable to FDA." They also give a sponsor the option to use other procedures but caution "... the sponsor to discuss the propriety of the alternative procedures in advance with FDA to prevent the expenditure of money and effort on activity that may later be deemed to be unacceptable." The CVM is also proposing a document regarding "Target Animal Safety Guidelines for New Animal Drugs." This document addresses guidelines for safety determinations of new animal drugs in all animals for which a new drug may be intended. The CVM says in the introductory section that " These guidelines...should remain flexible to allow scientific discretion in the design and execution of studies which will yield the maximum information on a product." The guideline also recommends that "the protocol be submitted...before the trial begins."

In a draft of "Contact Lens Product Guidelines" the Center for Devices and Radiological Health (CDRH) has suggested protocols for studies to provide data to fulfill requirements for toxicological testing, chemical testing, microbiological tests and for clinical studies of contact lense products. They also list some possible tests that provide alternatives to the use of animals and encourage efforts to continue the development of such tests. The CDRH says that the guidelines have been designed to answer most preliminary questions, but emphasizes "...that each potential applicant (IDE or PMA) for a contact lens product should consult with the Division of Opthalmic Devices (CDRH) prior to the start of any tests if unusual situations arise or if the sponsor has specific questions about the study design. This consultation is sure to clarify the pertinent requirements and to simplify the process of compiling your application."

There are "Guidelines for Preclinical Toxicity Testing of Investigational Drugs for Human Use" issued by the Center for Drugs and Biologics (CDB). These contain recommendations for the types of toxicologic studies in laboratory animals which must precede the various phases of clinical investigation of new drugs. With these guidelines, a major objective is to get the maximum amount of information with the minimum number of tests.

Efforts are continually made to improve present test requirements and there are examples of modifications resulting in the use of fewer animals. The CVM has modified a test to determine animal drug tolerance in a way which has reduced the number of animals per test from 20 to not more than four. CDRH has made revisions over the past 10-12 years in guidelines for assessing potential toxicity of contact lenses which resulted in reducing the number of animals per test from 72 to 12. And a final example, CDB has replaced animals completely with chemical tests for determining potency of some biological products.

The review of testing and research requirements and procedures has shown that the process of re-evaluating and improving tests as the science and knowledge base improves has been on-going for a number of years. As a result, there have been reductions in the numbers of animals used in some tests, elimination of the need for animals in some tests and a formalization of research and testing guidelines - all of which contribute to an overall effort to derive the maximum benefit from the minimum use of animals.

ISSUE #2 - Do FDA procedures in any way indirectly stimulate the perpetuation of the LD_{50} test even though the agency no longer directly requires the use of this test?

FINDINGS:

As can been seen from Tables 1-9, there are many testing procedures required throughout the Agency to characterize the toxic properties of chemicals, and it is clear that, in general, they do not directly or indirectly perpetuate the use of the traditional LD_{50} test.

At a workshop on acute studies, sponsored by FDA, on November 9, 1983, the conclusion was reached and a statement made that FDA has no regulations requiring use of the LD_{50} test. It was also stated that an approximation of this value is sufficient for all except a few highly toxic drugs such as some cancer chemotherapeutic agents. However, during this study, the Steering Committee found that there is a Code of Federal Regulations (21 CFR Part 450) requirement that each of three antitumor antibiotics, because of their inherent toxicity, have LD_{50} data prior to batch release. The Committee also learned that the Agency is considering eliminating this requirement. Several instances were found where references to the LD_{50} test still exist, even though there are no existing requirements for the test. In every case, changes are being made in order to make the Agency position absolutely clear.

A reference to LD_{50} in the preamble to the Good Laboratory Practice (GLP) Regulations (43 FR 59986) was intended to clarify that if the test were done, it was subject to the regulations since data from the tests "...may serve as part of the basis for approval." This may have been misinterpreted to mean that the Agency requires the test. The preamble is being revised to clarify the meaning.

"Guidelines for Preclinical Toxicity Testing of Investigational Drugs for Human Use," which have been in existence for many years, contain references to LD_{50} values although with emphasis on the greater need to characterize the dose and time relationships of toxic effects. The guidelines will be revised to state that LD_{50} values presently specified for drug combinations may be approximated from an acute toxicity study, rather than derived from a classical LD_{50} test.

The Code of Federal Regulations (21 CFR 202) specifies information for physician labeling of prescription drugs. An overdosage section includes oral LD_{50} values, if available, but the value need not be statistically precise and is often derived from an acute study.

The CFSAN guide on "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives used in Food" provides guidance for the conduct of oral LD_{50} studies. However, the guide emphasizes that this is not a required test and that "LD_{50} values are not as useful as other indices of toxicity derived from acute toxicity studies." The test protocol is included for use in the rare event no other test will suffice.

The CDRH draft guidelines for contact lens products require acute toxicity data on contact lens solutions and state that submission of LD_{50} data is one of several ways to fulfill this requirement. The Center has determined that appropriate safety information can be obtained from an acute oral study of contact lens solutions without the need for a traditional LD_{50}, and final guidelines will reflect this decision.

In addition to these specific references, the Steering Committee found that there may be instances where Agency and industry scientists use the term "LD_{50}" when they actually mean acute toxicity studies. The casual misuse of this term may be a contributing factor in the misunderstanding of FDA requirements. Some confusion may also result from the fact that when the FDA, through its National Center for Toxicological Research (NCTR), conducts tests for other agencies these tests may involve LD_{50} determinations to meet someone else's legal requirements.

The Centers have begun to take steps to resolve any misunderstanding in terms. In addition, most older guidelines have been or are being rewritten. Through review mechanisms in place and current heightened awareness on the part of Agency personnel, written requirements describing new or revised guidelines will reflect the position that use of this test should be avoided except for those rare situations where no alternative exists.

ISSUE #3 - Is FDA making maximum use of and encouraging the continued development of reliable *in vitro* alternatives to *in vivo* methodologies?

FINDINGS

Every Center within FDA has been involved for a number of years in the development and assessment of alternative approaches to reducing the use of animals. There are specific instances where requirements for animal tests have been eliminated or are being considered for elimination as the reliability of alternative procedures is validated. For example, cell culture systems have been shown to be equally or more sensitive than mice, guinea pigs and rabbits in tests for extraneous microbial agents that may be present in inactivated products such as poliomyelitis and rabies vaccines and for similar tests of live virus vaccines such as measles, mumps, rubella and the oral poliovirus vaccines. Appropriate changes in the current additional standards for these biological products will be made to delete the requirement for the use of animals in testing. Also the use of cell cultures for testing the presence of residual live virus in inactivated poliomyelitis vaccine is being evaluated to determine if they are as reliable as monkeys. Preliminary results indicate that the cell culture systems may be more sensitive. For medical device products, approval has been given for industry to substitute a variety of chemical and cell culture tests for *in vivo* tests of material toxicity and identification and for quality control. Pyrogen testing of drug products and biological products is changing from using rabbits to using the Limulus Amebocyte Lysate (LAL) assay to determine the presence of bacterial endotoxins. Guidelines addressing this change have been proposed, and comments received on them are currently being reviewed. In fact, some manufacturers have already received approval to substitute LAL tests for the use of rabbits. Attempts are being made to develop *in vitro* methods to replace animal tests presently used for assaying foods for protein quality (PER) and vitamin D content.

Immunochemical and biochemical techniques are being substituted for animals to determine the potency and purity of some biological products. Analytical methodology such as spectrophotometry is used to assure potency of meningococcal and pneumococcal polysaccharide vaccines and chromatography is used to determine the identity and molecular configurations of new products using recombinant DNA technology. Single radial immunodiffusion procedures are used to determine the potency of influenza vaccines and are also currently being evaluated for determining the potency of rabies and inactivated poliomyelitis vaccines. The utility of enzyme-linked immunoassay (ELISA) and radioimmunoassay (RIA) is also being evaluated as a suitable replacement for potency testing of poliomyelitis vaccines which currently requires the use of monkeys.

Research and development of a number of other alternative methods is being conducted or supported. Tissue culture, cell culture and subcellular cultures are being evaluated for the application to test for many substances such as heparin and protamine sulfate. Genetic probes, developed through advances in recombinant DNA technology, are being investigated for their application in assessing virulence and pathogenicity of food borne bacteria. Probes are now available for *Escherichia coli*, and *Yersina enterocolitica* with probes under development for *Shigellae*, *Campylobacter jejuni*, *Clostridium perfringens*, *Bacillus cereus*, *Vibrio cholera*, *Salmonella* and *Clostridium botulinum.* The Agency is following studies of cell culture methods using corneal epithelial stromal and endothelial cell lines and use of a protozoan species as alternatives to the use of animals for identification of ocular irritants.

For cosmetic ingredients, *in vitro* tests using ocular tissue cultures and cadaver skin in the Franz cell are now used frequently to provide information on skin sensitization and percutaneous absorption of cosmetic ingredients.

Unscheduled DNA synthesis, mammalian cell transformation, mouse lymphoma and the Ames Salmonella Reversion test are being investigated for their value in providing information on food additive and contaminant toxicity.

Scientists at the NCTR also use *in vitro* methods and procedures for a variety of research purposes. These include primary hepatocyte cultures for metabolism studies and the Chinese hamster ovary cells and the Ames test to determine mutagenic effects. Microorganisms such as bacteria, yeasts and fungi, are being used instead of animals to assess the toxicity of environmentally important chemicals.

Agency scientists are keeping abreast of activities outside FDA through attendance at scientific meetings, workshops, review of scientific literature, and professional interaction with other scientists in academia and industry. Some scientists serve as members of advisory panels or as primary consultants to professional societies or other organizations involved in studying the use of alternative methods such as the Society of Toxicology and the Johns-Hopkins Center for Alternatives to Animal Testing.

The Agency as a whole is actively keeping abreast of and considering non-animal models for use in both research and testing. However, it is not likely that requirements for the use of animals will be eliminated soon. Efforts to reduce the use of animals, while still providing sufficient data to evaluate the toxicity of compounds, will continue at as great a pace as scientific developments justify.

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ISSUE #4 - Are mechanisms in place to ensure continuing compliance with the Animal Welfare Act and with the highest standards of animal care?

FINDINGS:

The FDA's laboratory practices comply with the Animal Welfare Act as well as with other standards for humane care and use of animals. All Centers have acceptable procedures, but they vary from Center to Center in specific details. For example, two facilities have full accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and other facilities have acceptable self-assessment procedures for assuring proper animal care.

Accreditation by AAALAC is sought on a voluntary basis because it represents the highest form of approval for laboratory standards for animal care. It involves a visit and evaluation by experts in laboratory animal science who submit a detailed report to the Council on Accreditation. Accredited facilities submit annual status reports and are site-visited at least every three years. Full accreditation is accepted by the National Institutes of Health as assurance that the animal facilities are evaluated in accordance with Public Health Service (PHS) policy. In addition, there are procedures, other than through AAALAC accreditation, for assuring adherence to proper animal management practices also accepted by the PHS as appropriate and adequate. This includes assurance by a responsible official that there has been a self-assessment and the facility (1) accepts as mandatory the "Principles for the Care and Use of Laboratory Animals," (2) is committed to implementing the recommendations contained in the "Guide for the Care and Use of Laboratory Animals," and (3) is complying with the Animal Welfare Act and all other applicable federal statutes and regulations. Although not AAALAC accredited, the other FDA laboratories follow these PHS standards as well as FDA's GLP regulations. Examples of the types of mechanisms the various Centers utilize to ensure high standards of animal care are discussed below.

The animal facilities serving the Office of Biologics Research and Review (OBRR) in the CDB and the facilities of the NCTR are both fully accredited by AAALAC. Both have formal procedures for informing their staff of the policies on the care and use of animals. Among other things, NCTR and OBRR have adopted an "Animal Use Form for Experimental Protocols" and require every investigator using animals to provide a Committee on Care and Use of Animals with detailed information for evaluation of the protocol. Investigators are required to inform the Committee of any changes in the protocol which may be required during the course of the project.

CVM has an Animal Welfare Committee that provides general oversight in the planning and conduct of intramural research. CVM requires that study designs be reviewed and approved before a project is initiated and that all nonclinical projects be monitored in accordance with an established quality assurance program. The staff, which includes two veterinarians certified by the American College of Laboratory Animal Medicine is well qualified. Some staff have received American Association of Laboratory Animal Science (AALAS) technician training and others have had in-house training in the proper care and handling of animals. The Center is moving toward AAALAC accreditation.

The Division of Toxicology in the CFSAN also has a protocol review committee which reviews studies for compliance with established guidelines prior to commencement and the CFSAN has a quality assurance unit which monitors all the Center's laboratory studies. Two veterinary medical officers on the staff are responsible for assuring proper animal care.

CDRH and the Office of Drug Research and Review (ODRR), CDB, both conduct relatively limited animal research and therefore monitor their work differently from the other Centers. CDRH utilizes the AAALAC accredited animal welfare committee in the ODRR, CDB, to provide oversight and assistance. The ODRR, CDB, has no formal committee, but assures, through responsible supervisors, that studies are conducted in conformance with appropriate standards for animal care.

With regard to extramural programs, the Agency requires that all awardee institutions abide by written PHS policy and procedures. This includes (1) having in place a program of animal care which meets federal and Department standards, (2) providing through AAALAC accreditation or defined self-assessment procedures assurance of institutional conformance, and (3) maintaining an animal research committee to provide oversight of the institution's animal program, facilities and associated activities.

In summary, the FDA has procedures for assuring that its intramural and extramural programs and practices comply with high standards for animal care and welfare. By virtue of the nature of their program requirements and the amount of research or testing involving the use of animals, some Centers have more formal procedures than others and more veterinary staff capabilities. The Agency will continue to assure adherence to appropriate standards and will continue to improve facilities and procedures to establish and maintain superior standards throughout the organization.

ISSUE #5 - Is the historical usefulness of animal testing in human health protection, the primary mission of FDA, properly appreciated by our constituents?

FINDINGS:

As indicated in the discussions of the Steering Committee findings on the first four issues, FDA practices and procedures demonstrate appropriate and humane use of animals and the Agency supports the development of alternative tests. Development and evaluation of procedures to minimize the use of animals is a continual process. However, it is a fact that the use of animals has been and continues to be essential to determine the safety of products regulated by FDA. It is important that this requirement be recognized and understood along with the importance of promoting proper use of animals.

The FDA uses a number of mechanisms for communicating its need to use animals in fulfilling its responsibilities to protect public health. These include attendance and participation by Agency personnel in meetings, workshops, conferences, symposia, etc., which provide opportunities to discuss FDA responsibilities, requirements and actions. In addition, FDA, through Talk Papers and publications such as the FDA Consumer and the FDA Veterinarian, reaches other segments of the public to inform them of FDA activities. The Office of Legislation and Information responds to Congressional inquiries in these areas. Through the Office of Science Coordination, FDA has been responding to public inquiries (as has the Office of Consumer Affairs) and has been interacting with the Office of Technology Assessment in their assessment of "Alternatives to Animal Use in Testing and Experimentation."

The methods of communication mentioned above primarily reach the public at large and are useful and important. Just as important, however, is the issuance of guidelines describing testing requirements and protocols. These are essential to industry and in most cases provide a rationale for the requirement.

Although these mechanisms do not focus exclusively on animal welfare, they are well established procedures for communicating with the broad range of FDA constituents. It is difficult to assess formally how successful FDA has been in creating an awareness of the essential role animals play, but results of polls over the past several years indicate a high degree of public awareness and approval of the Agency's role in both human and animal health protection.

Through the channels mentioned above and through a renewed, concerted effort to be certain that industry understands Agency requirements, FDA will continue to place the use of animals in proper perspective. It will also continue its policy to improve the welfare of animals and to examine its requirements in an effort to reduce the numbers of animals needed.

Recommendations

As a result of its review of Agency practices and procedures, the Steering Committee has the following recommendations.

1. Under the sponsorship of the Office of the Commissioner, organize and conduct a series of workshops addressing the following issues:

   a. Acute toxicity studies required throughout the Agency

   This would be attended by research and regulatory staff and deal with requirements for sponsors and for FDA staff. The objective would be to assure that everyone uses the same terms in dealing with industry and also to inform staff members from each Center of the requirements in other Centers.

   b. Use of in vitro alternatives by various Centers

   The focus would be on the science, but an objective would also be to make staff members in each Center aware of the way other Centers utilize in vitro methodologies. The Committee found that a number of unique methods are under development and also that some of the same basic methods are being used in different Centers for different purposes. An exchange of information and views would strengthen the Agency science base in this area.

   c. Agency and PHS practices and procedures for the care and handling of animals

   As with the other areas, practices vary from Center to Center and Agency staff members can benefit by sharing information. It would also provide an opportunity to inform staff members of developments at NIH and in some other agencies since FDA participates with them in the area of animal welfare.

2. Establish an Agency-wide animal welfare committee. The committee would be interdisciplinary and function as a resource to the various Centers and to the Commissioner. It would not have oversight responsibilities, but would be advisory in nature.

TABLE 1

CATEGORY: DIRECT FOOD ADDITIVES AND COLOR ADDITIVES* AND INDIRECT FOOD ADDITIVES

PURPOSE: Petition, Regulatory support

Study	Test Period	Animals Used
Acute oral	7-14 ds.	rodent
Short term oral (cont. exposure)	28 ds.	rodent
Subchronic oral	90 ds. 90 ds.	rodent non-rodent
Chronic	12 mo. 12 mo.	rodent non-rodent
Carcinogenic potential	short term	variable
Carcinogenicity	24 mo.	rodent
Combined Chronic/Carcinogenicity	24 mo.	rodent
Teratogenicity	6-18 ds.	rodent, rabbit
Reproduction w/teratology phase	multigeneration	rodent
Absorption, distribution, metabolism & elimination	test dependent	test dep.
Neuro-behavioral	test dependent	rodent, rabbit
Immunotoxicity	test dependent	rodent

* For other than food colors, special tests would be required. Examples are colors used (a) in cosmetics in the area of the eye, (b) in contact with mucous membranes, (c) in sutures, and (d) in parenteral solutions.

TABLE 2

CATEGORY: COSMETICS

PURPOSE: Industry Guidance and Regulatory Support

Study	Test Period	Animals Used
Acute oral	7-14 da.	rodent
One hour inhalation	1 hr.	rodent
Acute inhalation	14 da.	rodent
Eye irritation	7-14 da.	rabbit
Dermal irritation	7-14 da.	rabbit
Primary skin & corrosivity	7-14 da.	rabbit
Skin sensitization	7-14	guinea pig
Phototoxicity	1-3 da.	nude mouse, rabbit, guinea pig

TABLE 3

CATEGORY: NUTRITION

PURPOSE: Nutrition labeling, regulatory support

Study	Test Period	Animals Used
Protein Quality	28 ds.	rodent
Vitamin D Bioassay	18-25 ds.	rodent

TABLE 4

CATEGORY: CHEMICAL, BIOLOGICAL CONTAMINANTS

PURPOSE: Regulatory support

Study	Test Period	Animals Used
Subchronic	90 ds. 90 ds.	min. swine dog
Chronic	12 mo.	dog
Seafood toxin assays		rodent
Microbiological Assays		rodent, rabbit
Chemical contam. assays	test dependent	rodent
Metabolism	test dependent	test dep.
Neuro-behavioral	test dependent	rodent, rabbit
Immunotoxicity	test dependent	rodent

TABLE 5

CATEGORY: OPTHALMIC DEVICES, OTHER DEVICES AND RADIOLOGICAL PRODUCTS

PURPOSE: Petition, Regulatory support

Study	Test Period	Animals Used
Ocular irritation & corneal metabolism (Class III contact lens materials & solutions)	3 wk.	rabbits
Sensitization Study (Class III opthmalic products for intraocular use)	7-14 ds.	guinea pig
Acute Systemic toxicity (Class III opthalmic device products)	4 ds.	rodent
Color additives (Class III opthalmic device products)	Same tests as for color additives (see Table 1).	
Biomaterial implant study (Class III opthalmic products for intraocular use)	variable	rabbit, primate cat
Acute oral toxicity (Class III contact lens solutions)	14 ds.	rodent
Primary ocular irritation (Class III contact lens accessory products)	3 ds.	rabbit
USP Intracutaneous test (Class III opthalmic products for intraocular use)	3 ds.	rabbit
Other devices & radiological products	Tests high dependent on device/ product and intended use. Determined on case-by-case basis.	

TABLE 6

CATEGORY: NEW VETERINARY DRUGS

PURPOSE: Petitions, regulatory support

Study	Test Period	Species*
Safety, Efficacy	use dependent	target species
Drug tolerance	1-3 wks.	target species
Repro. studies	species/test dep.	target species
Tissue irritation	drug dependent	target species
Combination drug	comb. dependent	target species
Drug disposition	test dependent	target species
Route of admin.	drug dependent	target species
Intramammary infusion	8-10 ds.	dairy cows, goats
Behavioral	-	target species

* Target species is the animal in which drug is to be used.

TABLE 7

CATEGORY: NEW VETERINARY DRUGS, FOOD PRODUCING ANIMALS

PURPOSE: Petitions, regulatory support

Study	Test Period	Species*
Subchronic oral	90 da.	nonrodent rodent
Chronic oral	6 mo.	rodent
Chronic oral	12 mo.	nonrodent
Carcinogenicity/comb. chronic	2 yr.	rodent
Hormonal	6 mo.	monkey
Carcinogenicity	2 yr.	rodent
Teratogenicity	6-18 da.	rodent
Reproduction/teratogenicity	2 generations	rodent
Special studies (neurotoxicity, cardiovascular, behavioral, etc.)	test dependent	test dep.
Carcinogenic potential	in vitro	variable

GORY: NEW HUMAN DRUGS

PURPOSE: Drug application, regulatory support

ANIMAL STUDIES REQUIRED (USUALLY BY PROPOSED HUMAN ROUTE)*

Expected Therapeutic Use	Before initial (Phase I) human tolerance study	Before early (Phase II) human safety/efficacy study	Before including women of childbearing potential	Before extended (Phase III) safety/efficacy study	Before Marketing
1 - few days (e.g., anesthetics, diagnostics (usually) non-oral)	↑	none further	↑	none further	none further
Limited use (days-weeks) or patient population (e.g., most parenterals & local-use topicals)	**Acute tox.** Rod. Non-rod. **Subchronic tox.** (2-13 wk.) Rod. Non-rod. **Irritation** (parenterals & topicals) Rabbit **Pharm. & Metab.** Rod. variable Nonrod.	↑ Longer subchronic tox.** (to 13 wk.) if needed rod. nonrod.	**Terat.** Rod. &/ rabbit **Other repro.** as approp. rod.	None further	none further
Unlimited use (chronic repeated, widespread) e.g., most oral drugs, some inhalation drugs and certain topicals if absorbed and/or extensively used	↓	↓	↓	**Chronic tox.** (underway) Rod. Non-rod. **Carcinogenicity** (underway) Rod. Non-rod (oral contraceptives only)	none further (chronic tox. & carcinog. studies completed

* Parenteral acute toxicity is sometimes done for oral drugs (to estimate absorption) and oral studies are sometimes done for topical or inhalation drugs.

** Usually study duration appropriate to phase II (i.e., up to 13 weeks) precedes phase I.

TABLE 9

CATEGORY: Biologic Products
PURPOSE: Product Licensing, Product Lot Release

PRODUCT	TYPE	Test Period	ANIMALS USED
All biologics administered by injection	General Safety	7 ds.	Guinea pigs Mice
	Pyrogenicity	4 hrs.	Rabbits
Anthrax Vaccine	Potency	24 ds.	Guinea pigs
BCG Vaccine	Hypersensitivity	4-6 wks.	Guinea pigs
	Potency	6 wks.	Guinea pigs
Botulism Antitoxin	Potency	7 ds.	Mice
Cholera Vaccine	Toxicity	72 hrs.	Mice
	Potency	14-18 ds.	Mice
Diphtheria Toxin	Potency	40-66 hrs.	Mice
Diphtheria Toxoid	Potency	5 wks.	Guinea pigs
Diphtheria Antitoxin	Potency	7 ds.	Guinea pigs
Pertussis Vaccine	Toxicity	7 ds.	Mice
	Potency	28-31 ds.	Mice
Plague Vaccine	Potency	4 wks.	Mice
Tetanus Toxoid	Potency	5-7 wks.	Guinea pigs
Tetanus Immune Globulin	Potency	7 ds.	Guinea pigs
Tuberculin	Safety	6 wks.	Guinea pigs
	Potency	18-24 hrs.	Guinea pigs

TABLE 9 - CONTINUED

PRODUCT	TYPE	Test Period	ANIMALS USED*
Typhoid Vaccine	Potency	10-17 ds.	Mice
Immune Serum Globulin	Potency	7 ds.	Guinea pigs
Hepatitis B Vaccine	Safety	21 ds.	Mice
	Safety	14 ds.	Suckling mice
	Safety	24 wks.	Chimpanzees
	Potency	28 ds.	Mice
Measles Virus Vaccine	Safety	21 ds.	Mice
	Safety	14 ds.	Suckling mice
	Safety	17-21 ds.	Monkeys
Mumps Virus Vaccine	Safety	21 ds.	Mice
	Safety	14 ds.	Suckling mice
	Safety	17-21 ds.	Monkeys
Measles Virus Vaccine	Safety	21 ds.	Mice
	Safety	14 ds.	Suckling mice
	Safety	17-21 ds.	Monkeys
Poliomyelitis Vaccine	Safety	21 ds.	Mice
	Safety	17-19 ds.	Monkeys
	Potency	21 ds.	Monkeys
Poliovirus Vaccine	Safety	42 ds.	Guinea pigs
	Safety	21 ds.	Mice
	Safety	17-21 ds.	Monkeys
	Safety	3 wks.	Rabbits
	Safety	14 ds.	Suckling mice
Rabies Vaccine	Safety	21 ds.	Mice
	Potency	28 ds.	Mice

AGENCY STEERING COMITTEE ON ANIMAL WELFARE ISSUES

Dr. Allen Heim, Chairman	Office of Science Coordination
Dr. Arnold Borsetti, Vice Chairman	Office of Science Coordination
Dr. Richard Bradbury	Center for Veterinary Medicine
Dr. Mary Ann Danello	Special Assistant for Science, Office of the Commissioner
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Dr. Attila Kadar	Office of Regulatory Affairs
Dr. Kenneth Krell	Center for Devices and Radiological Health
Dr. Neil Littlefield	National Center for Toxicological Research
Dr. Robert Osterberg	Center for Veterinary Medicine
Dr. Moris Shore	Center for Devices and Radiological Health
Dr. James Vickers	Center for Drugs and Biologics
Mrs. Peggy Perry, Executive Secretary	Office of Science Coordination

Dr. **Guest**. As a part of its overall effort to reduce or avoid unnecessary testing methods, FDA has been carefully examining the use of the Draize eye irritant test which utilizes test rabbits. Unfortunately, the Draize test is still the most reliable method for determining the potential harmfulness, or safety of a product instilled in the eye, such as ophthalmic drugs and devices and some cosmetic products.

FDA is considering alternatives to the rabbit eye test and to other animal protocols. Among the assays which show promise of replacing the Draize test are cell culture methods using cornea and other cell lines. In addition, in vitro cell culture research using a protozoan species as a model for identifying ocular irritants and tissue culture method utilizing excised cornea from animals or eyebank eyes are being investigated.

It is my understanding that a number of toxicological laboratories are now involved in testing a battery of sensitive in vitro assays reported to be useful in ranking as mild to severe irritants. However, these assays need further development and cannot fully yet replace the Draize test.

Complete validation of an assay requires that it be tested on a wide spectrum of compounds, in many different laboratories. In vitro findings must be related to in vivo and the results must indicate that the assay is predictable, reliable, and reproducible.

Again, Drs. Rall and Willett have already commented extensively that is going on and is planned at NIH to reduce animal use. We have a number of those things going on at the Food and Drug Administration, and rather than detail that at this point I would like to submit that for the record at a later time.

[The information follows:]

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SUBMISSION FOR THE RECORD

Subcommittee on Science, Research
and Technology
Committee on Science and Technology
House of Representatives

May 6, 1986

National Center for Toxicological Research

It is generally considered that chemical carcinogens act through a mechanism which includes damage to the DNA of the exposed organism. These types of chemicals have been called genotoxins, i.e., toxic to the genome. A multitude of short-term bioassays have been developed in an attempt to detect these types of carcinogens including bacterial and somatic cells in culture. The NCTR has used several of these systems to help predict the potential carcinogenicity of a chemical; these include the Ames Salmonella/microsome system; the Chinese hamster ovary hypoxanthine quanine phosphoribosyl transferase system (CHO/HGPRT); the CHO/sister chromatid assay (CHO/SCE); the primary hepatocyte/DNA repair system and gut flora metabolic activation. These assay systems are *in vitro* systems and do not use treated animals and are useful only in *so far* as our present understanding of how genotoxic carcinogens act. All of these systems can be utilized solely as *in vitro* systems or can be coupled to the whole animal in an *in vivo*/*in vitro* approach.

Ames Salmonella/microsome assay:

This assay is dependent upon reaction of a chemical or metabolite with the DNA of a specially constructed bacterium. The DNA damage produced may be transmitted as a mutation to the progeny of the treated parents. This mutation can be quantified as the ability of the cells to grow in a medium devoid of the amino acid histidine. When a population of these bacteria are treated with genotoxic carcinogens the response is usually dose-dependent. Many of the mutagens detected in this system have been identified as animal carcinogens, hence its applicability to predicting the carcinogenic process. This system is generally insensitive to non-genotoxic carcinogens.

CHO/HGPRT assay:

This assay is dependent upon reaction of a chemical or metabolite with the DNA of the CHO cell grown in a tissue culture environment. The DNA damage produced may be transmitted as a mutation to the progeny of the treated parents. This mutation can be quantified as the ability of the cells to grow in a medium containing the quanine analog 6-thioquanine; cells resistant to this analog have lost the purine salvage pathway enzyme HGPRT. When a population of these mammalian somatic cells are treated with genotoxic carcinogens the response is usually dose-dependent. Many of the mutagens detected in this system have been identified as animal carcinogens, hence its applicability to predicting the carcinogenic process. This system is used to complement the above system and is thought to have more relevance because the genome studied is mammalian and not bacterial in origin and theoretically would be more predictive of the animal bioassay and from there the human. This system is generally insensitive to non-genotoxic carcinogens.

CHO/SCE assay:

This assay is considered to be dependent upon reaction of a chemical or metabolite with the DNA of the CHO cell grown in a tissue culture environment. It has also been shown, however, that alterations in nucleic acid pools can lead to increases in SCEs and suggests that perhaps carcinogens which effect pool size will be detected. The assay is dependent upon the ability to incorporate bromodeoxyuridine (BrdU) into the chromosome through two cell cycles and then differentiating the sister chromatids. It has been observed that genotoxic carcinogens increase exchange between sister chromatids in a dose-dependent manner, hence its applicability to predicting the carcinogenic process. Unlike mutations, the relationship of induction of SCEs to carcinogenesis is less well understood. This system is highly sensitive and has been shown to detect both genotoxins and some non-genotoxins. A further advantage of this system is that any cell which replicates can be used including lymphocyte of animals on chronic bioassay or lymphocyte from humans in high risk occupations or humans exposed to potential genotoxins.

Primary hepatocyte/DNA repair system:

This assay is dependent upon reaction of a chemical or metabolite with the DNA of a primary hepatocyte cultured in vitro tissue culture environment. The assay is dependent upon the incorporation of radiolabeled thymidine, a precursor to DNA synthesis, into DNA during the G0, G1, G2 part of the cell cycle and is referred to as unscheduled DNA synthesis (UDS). It has been observed that many genotoxic carcinogens induce UDS in hepatocytes in a dose-dependent manner, hence its applicability to predicting the carcinogenic process. The advantage to using hepatocytes as the target organism is related to the ability of this intact cell to metabolize a variety of xenobiotics in vitro.

Gut flora metabolic activation:

An impressive array of enzymatic reactions can be performed by the intestinal microflora on both endogenous and exogenous compounds. These reactions both complement and antagonize those carried out by the liver. An in vitro semi-continuous culture system that simulates the human large intestine has been developed at the NCTR to determine the role of intestinal microflora in the metabolic activtion of potential carcinogens. This approach is an interesting alternative to traditional methods, e.g., laboratory animals and bacterial monocultures or suspensions and a new tool in defining the toxicological role of the intestinal microflora.

Dr. GUEST. Our hope is that these new tests will result in the reduction of animals currently used in the whole animal tests.

In summary, Mr. Chairman, in recent years we have been making a concerted effort to avoid unnecessary or obsolete animal tests, to reduce the number of animals required in tests and to develop in vitro alternatives whenever possible. It is, of course, the agency's primary mission to protect the consumer in the drugs and foods area, and in carrying out this mission this agency must recommend testing procedures which have been universally recognized as valid for detecting any ocular of an ophthalmic drug product prior to its human use.

The agency regrets the necessity of animals being used for toxicological testing and has taken steps to promote humane treatment of these animals as well to minimize the numbers of animals used in testing and in research.

I will be pleased to answer any questions that you may have, sir.

[The prepared statement of Dr. Guest follows:]

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

STATEMENT BY

GERALD B. GUEST, D.V.M.

ACTING DIRECTOR, CENTER FOR VETERINARY MEDICINE

FOOD AND DRUG ADMINISTRATION

PUBLIC HEALTH SERVICE

DEPARTMENT OF HEALTH AND HUMAN SERVICES

BEFORE THE

SUBCOMMITTEE ON SCIENCE, RESEARCH AND TECHNOLOGY

COMMITTEE ON SCIENCE AND TECHNOLOGY

HOUSE OF REPRESENTATIVES

MAY 6, 1986

FOR RELEASE ONLY UPON DELIVERY

Mr. Chairman:

The Food and Drug Administration (FDA), like other components of the Department of Health and Human Services, is actively pursuing a number of initiatives to insure the most humane treatment possible for test animals and to reduce or eliminate entirely requirements for such tests as the Draize and LD_{50}.

In their testimony this morning, Drs. Rall and Willett of the National Institutes of Health (NIH) present excellent overviews of Department efforts to minimize the use of animal tests. They correctly emphasize, however, that since animals are the best surrogates for humans, there will be a continued need for their use in research in order to minimize risk to human health. This need is reflected in the principal law administered by FDA, the Federal Food, Drug, and Cosmetic(FDC) Act.

FDC Act Requirements

The Act imposes on manufacturers the burden of demonstrating that their products meet the safety requirements of the law. Apart from the work done by the National Center for Toxicological Research in Arkansas, FDA conducts relatively little toxicology testing of its own. Instead, we recommend the type and extent of testing we believe necessary for a determination of safety -- and then review the data submitted by manufacturers to determine whether they meet these requirements.

Thus, we require that all new human and animal drugs and food additives undergo careful testing in animals:

- -- to assess their potential toxicity in humans;
- -- to determine whether they have any teratogenic potential; and
- -- to determine carcinogenicity whenever there is the likelihood of chronic exposure of humans.

Animal studies of human drugs are of particular importance in determining whether new products can safely be tested in humans to assess their potential therapeutic effect. Obviously, it would be neither legal nor ethical to begin human trials of a drug until its acute toxicity and other harmful potential effects have been carefully tested in animals.

Chemicals used as drugs to treat animals or as animal feed additives require toxicity testing for several reasons. One reason is the requirement to assure safe use in the target animal whether it be a food producing animal such as poultry or cattle or non-food animals such as horses, cats and dogs. Another is the necessity to assure that those chemicals used in food producing animals are safe if they become a component of human food as a residue.

Despite the need for animal testing to some degree, FDA has already undertaken or plans to undertake efforts to reduce the use of animals in research and testing and to avoid unnecessary testing methods.

Acute Studies Workshop

On November 9, 1983, FDA sponsored an acute studies workshop atter by approximately 150 persons from Government, industry and public interest groups. The purpose of the workshop was to discuss the scientific rationale, requirements and uses for acute toxicity stud including lethality, to clarify the regulatory requirements for acu toxicity data and whether there was any longer a need for a statistically exact LD_{50} value, or the dose which kills 50 percent of a group of the laboratory animals under study.

The following consensus points emerged from the workshop:

-- There was general agreement among Government and industry representatives that the LD_{50} test is often credited with greater quantitative and scientific accuracy than it merits an that there are other determinants of acute toxicity such as site and mechanism of action, early or delayed lethality and recovery rate that are better indices of toxicity and hazard than LD_{50} values per se.

-- The requirement for LD_{50} tests among Government agencies and industry is much less than that perceived by the general public. For example: FDA does not require the use of the LD_{50} test to assess the safety of the products it regulates.

-- Industry and Government agencies support the development and validation of alternative methods--those using as few animals as possible and those that use no animals.

-- United States Government agencies are cooperating on animal welfare issues with other countries through organizations such as the Organization for Economic Cooperation and Development (OECD).

FDA Steering Committee on Animal Welfare

As a result of this workshop, FDA established an Agency-wide Steering Committee on Animal Welfare Issues to review its guidelines on the use of animals and to recommend changes (where needed) to the Commissoner of Food and Drugs. On August 15, 1984, the Steering Committee issued its final report which called for, among other recommendations:

- -- greater coordination among FDA Centers in the use and development of in vitro alternatives to animal testing
- -- instituting more uniform Agency-wide practices for the care and handling of animals, and
- -- establishing a permanent FDA animal welfare committee.

The recommendations were adopted by the Agency and at present there is an active animal welfare committee. We would like to submit a copy of this Steering Committee's report for the record.

Draize Test

As part of its overall effort to reduce or avoid unnecessary testing methods, FDA has been carefully examining the use of the Draize eye irritant test which utilizes test rabbits. Unfortunately, the Draize test is still the most reliable method for determining the potential harmfulness, or safety, of a product instilled in the eye, such as ophthalmic drugs and devices and some cosmetic products. FDA is considering alternatives to the rabbit eye irritation test and to other animal protocols. Among the assays which show promise of replacing the Draize test are cell culture methods using corneal and other cell lines. In addition, in vitro cell culture research using a protozoan species as a model for identifying potential ocular irritants and a tissue culture method utilizing excised cornea from animal or eyebank eyes are being investigated. Four toxicology laboratories are involved in testing a battery of sensitive in vitro assays reported to be useful in ranking chemicals as mild to severe irritants. However, these assays need further development and cannot yet replace the Draize test. Complete validation of an assay requires that it be tested on a wide spectrum of compounds, in many different laboratories. In vitro findings must be related to in vivo data and the results must indicate that the assay is predictable, reliable, and reproducible.

Research Activities

Drs. Rall and Willett have already commented extensively on the research that is being done or planned at NIH and elsewhere to reduce the use of animals in testing. Rather than detail specific FDA activities at this time, I would like to submit for the record, a list of research activities which we have undertaken to develop new and appropriate tests which will either replace or refine existing tests. These we hope will result in a reduction of animals currently used in whole animal tests.

Conclusion

In summary, Mr. Chairman, in recent years we have been making a concerted effort to avoid unnecessary or obsolete animal tests, to reduce the numbers of animals required in tests and to develop *in vitro* alternatives whenever possible. I should remind the Subcommittee that the Agency's primary mission is consumer protection. In carrying out this mission, the Agency must recommend testing procedures which have been demonstrated to be universally recognized as valid for detecting any ocular irritancy of an ophthalmic drug product prior to human use. The Agency regrets the necessity of animals being used for toxicological testing and has taken steps to promote humane treatment of these animals as well as to minimize the number of animals used for testing and research.

We will be pleased to answer any questions you or the other Subcommittee members may have at this time.

Mr. **Walgren**. Thank you all for your testimony.

Is there some way where we can get some feel for the relative effort that is going into alternatives as opposed to the balance of effort that is ongoing in your respective shops?

Dr. Willett, your office is quite focused on alternatives, isn't it; is there other research going on there other than alternatives?

Dr. **Willett**. The section is focusing specifically on non-mammalian models in the cell and culture system.

Mr. **Walgren**. You said that it is about $1 million worth of effort?

Dr. **Willett**. That is just what this section is entailing in its activities in support of major resources in this area, things that are servicing the needs of a very wide range of the biomedical research community. The activities within NIH's research portfolio as a whole, that employ nonmammalian systems, are the kinds of things that you might refer to as alternative methodologies, is fairly large, some 27 percent of the entire portfolio has been that since as far back as I personally have been tracking it, which is 1977.

Mr. **Walgren**. Now, there we are talking about alternatives to mammals?

Dr. **Willett**. That term becomes very difficult to utilize in talking about the research domain, because usually when an investigator selects the model system for the study that they have in mind, the system they select is one that is suited to answering the question that they pose. It is difficult to ask the question, are there alternatives to animal models in research, or mammalian models in research, because you would use a mammalian model when that system was suited to your investigation.

Mr. **Walgren**. Or viewed as suited.

Do you know of situations where something maybe had been done that way, but that when someone comes along and proposes a new basis of the research, or a new model, a nonmammalian model, that they then have difficulty getting research with that in their protocol as opposed to the way it has always been done?

Dr. **Willett**. I have no real evidence for that. If you look at the use of cell and tissue culture systems as models across biomedical research since their first inception, I don't know how many years back that occurred, those items now are almost a routine tool within biomedical research as a whole. You will find almost any area within biomedical research that is looking at cell, cell function, physiology, whatever, utilizes that technology. So those model systems very rapidly were incorporated into the portfolio tools used by the research community.

Mr. **Walgren**. Has your office made awards in support of new model development?

Dr. **Willett**. No; our support so far has been exclusively for the American-type culture collection, and this MIT Cell culture center, and the partial support, and that has taken us to the limits of the funds we have available?

Mr. **Walgren**. Do those predate your office?

Dr. **Willett**. The American-type Culture Center did, that was supported by NIH funds at sort of the organizational level of the office of the director, and then those dollars were also moved to the division. The division had always had responsibility for administering the activity.

The MIT Cell Culture Center is a new activity.

Mr. **Walgren**. What is the dollar value of that?

Dr. **Willett**. That is about $256,000.

Mr. **Walgren**. And is there any other new activity, that you are supporting now?

Dr. **Willett**. Outside of the partial support, no, for the other activities that were mentioned. The Nematode Center at Missouri, which we partially support with the Aging Institute; the National Diabetes Research Interchange, which the division is sharing support with the Arthritis Institute; and the CDNA libraries which we support, sharing this activity with child health.

Mr. **Walgren**. What is the measure of new activities supported since the establishment of your office?

Dr. **Willett**. Through the section?

The new activities would be $256,000 with—roughly $500,000.

Mr. **Walgren**. Approximately $500,000—out of the total is $1 million at this point?

Dr. **Willett**. $1.1 million.

Mr. **Walgren**. Let me ask Dr. Rall, then can you respond to the same thought; how much new is going on in the area of alternatives? When you look at your budget and you break it up, are we talking about—what percentages are involved in the direct pursuit of alternatives?

Dr. **Rall**. There are a couple of ways of answering that. As I tried to point out, we have been deeply involved in that since the early 1970's. In fact, I chair, and was on the organizing committee of an effort that NCI, and NIGMS, and NHLBI sponsored a conference on comparative pharmacology, which really deals with much of this, back in 1967.

So our support has been growing, but it started at a fairly large level back in the early 1970's. It is somewhere between $10 and $15 million a year. That does not include the new request for application, that I mentioned, on nonmammalian species and toxicological testing.

Mr. **Walgren**. How much money is involved in that?

Dr. **Rall**. We don't know yet, this depends upon the quality of the grant applications and next year's budget, which we don't quite know yet. I would imagine a couple of million dollars, but that is a figure that I shouldn't be held to.

A couple of general observations. In science you look for the simplest system that works. And this actually forces science to move to nonmammalian systems, which tend to be simpler and also less expensive. And that is a constant pressure that I think is going on all the time.

Second the toxonomy isn't right to look for alternative methods. Nobody labels their research that way. So it becomes an almost impossible task to go into a large catalog of research efforts and pick out those.

In our toxiological efforts where we want to develop better methodologies we would describe it as development of new and better methodologies. If you ask for alternative tests, you wouldn't find it.

Mr. **Walgren**. Yes; but am I not right in thinking that the Congress has asked for your agencies to pursue this in particular, and although, when you look at the research that is done out there that is investigator-initiated, you might not see it leaping out as alternatives, nonetheless you are charged with administering a program that is designed to have a purpose, and one of those purposes is the development of alternatives. What effort can we show to the public that is targeted and designed to pursue that goal?

Dr. **Rall**. I think our RFA is precisely on. It was——

Mr. **Walgren**. RFA is that again?

Dr. **Rall**. Before this nonmammalian species, this is precisely what I think you all wanted. I discussed with some of the animal welfare people this precise project over the years.

Mr. **Walgren**. Yes; I think so, too.

What I am getting at and I don't want to be undercutting——

Dr. **Rall**. The other thing, we do this in the normal day-to-day business, we are always looking for better methods, and many of those are what would be called, currently alternate.

Mr. **Walgren**. Yes; but realizing that you have always done that, the question is one of how successful are we in finding it?

When we look at the budget of entity as a whole do we see that alternatives are thin layer of—whatever you call it—on top; or are they building in terms of that effort to occupy a reasonable amount of our budget?

Dr. **Rall**. In our MTP, which is around $70 million, they are about 20 percent. They have built up from about 8 percent over the past few years.

Mr. **Walgren**. Is a lot of that in the gene——

Dr. **Rall**. A lot of it is genetic toxicology, because some of the simpler systems are just beautifully available for that sort of stuff.

We also, of course, are interested in other things. The teratological effects, we are trying to study by using organ culture, and so forth, and so on.

Mr. **Walgren**. Let me ask you this, if you were to set aside the gene carcinogen tests, that 20 percent would be reduced substantially, would it be reduced to 3 percent, or 2 percent?

Dr. **Rall**. I don't know. I will go back and look.

Mr. **Walgren**. I would appreciate that.

Dr. **Rall**. It will be reduced substantially.

Mr. **Walgren**. Proportional measure; it is just a way of letting all of us know how much of an effort is going into one.

Dr. **Rall**. And we do expect this grant solicitation will bring forth many interesting ideas. I have scanned the applications and they should be a lot of fun.

We are, of course, now being—with this we will help support the Johns Hopkins and Rockefeller University effort.

Mr. **Walgren**. Well, then let me ask Dr. Guest, if I am not cutting you off, Dr. Rall, that this same measure at the FDA, is there any substantial proportion of funds being allocated to the pursuit of alternatives at this point?

Dr. **Guest**. I think we could furnish that.

[The information follows:]

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SUBMISSION FOR THE RECORD

Subcommittee on Science, Research
and Technology
Committee on Science and Technology
House of Representatives
May 6, 1986

FDA Resources Related to Pursuit of Alternatives to Animal Testing

Although FDA does not budget specifically for research on alternatives to animal testing, the Agency does perform research to develop test methods that are faster, more accurate and more reliable than animal testing so that FDA can better ensure that food and drug products are safe to consume. In fiscal year 1986, FDA will spend approximately $1.0 million, including 15 Full Time Equivalents (15 people working full time for one year each), in support of research on quality control methods that will substitute for currently used animal tests. Funding for the past three years has been approximately at that same level as well.

Center for Veterinary Medicine

Dirofilaria Immitis (Heartworm) Model:

The University of Pennsylvania was funded by FDA to develop an *in vitro* model to screen and evaluate the biochemical properties of compounds against precardiac and microfilarial stages of *D. Immitis*. Such a model may provide an alternative to using domestic animal species for preliminary evaluations against developing stages of filarial parasites.

Xenobiotic Metabolism Model:

The University of California at Davis was funded by FDA for the purpose of developing and validating an *in vitro* model that would both improve and expedite the process of assessing the hazard of drugs and chemicals in food animal species. The program objectives were to determine (1) whether an *in vitro* model based on isolated bovine hepatocytes is a biologically acceptable approach for studying xenobiotic metabolism (biotransformation and conjugation reactions) in bovine species, (2) whether the bovine hepatocyte is valid for predicting the generation of metabolites in the whole animal, and (3) the metabolic fate of selected chemical substances *in vivo* (cattle) and *in vitro* (isolated hepatocytes).

Center for Devices and Radiological Health

Peripheral Lymphocytes in Culture:

The use of the peripheral lymphocyte in short term *in vitro* cell cultures is a basic resource in the study of mammalian chromosomes, including humans, both to determine the chromosomal complement of an individual and to monitor changes in the chromosomes during some interval. Techniques have been developed to permit the use of peripheral lymphocytes to test individuals for responsiveness to mocrowave radiation. The test detects two types of changes: (1) blast transformation in which the lymphocytes enlarge and undergo a blast of DNA and RNA syntheses, and (2) an immune response in which immune markers increase on the surface of one or more types of lymphocytes. Both have been used to study responsiveness of different strains of mice. The two types of responses have not been correlated.

Sister Chromatid Exchange:

Cells in culture, including peripheral lymphocytes, can be examined after use of special strains to determine the frequency of exchanges between the two sister chromatids of the cells' chromosomes. Under some circumstances the appearance of sister chromatid exchanges parallels mutagenesis; a study correlating the two types of changes was performed after UV radiation exposure. At present, however, the test is viewed as useful for monitoring and for following up exposed individuals. As an indicator of genetic damage, the test should be used in combination with other genetic test systems. The sister chromatid exchange system may have potential use in monitoring leechates from implanted medical devices, and preliminary studies have been conducted with such an aim. A known problem for use of the exchange test in humans is that smokers react differently from non-smokers.

Corneal Cells in Culture:

Techniques have been developed and described for the separation of epithelial and endothelial cells of the cornea and for establishing cultures of both types of cells. The cell cultures have been used to study cell responses to ionizing and microwave radiations.

Mutagenesis Test System:

Substantial work has been performed to develop and test a cell line from a mouse lymphoma for quantitative mutagenesis studies of a variety of substances. The "Clive" strain of a standard line - L5178Y - has been used to test for mutagenicity of ultrasound, photosensitizing chemicals, selected other chemicals, and ultraviolet, and to compare the mutagenicity of a series of standard lamps. The test strain continues to be used widely among laboratories interested in genetic toxicity. Data derived with this test system have been used in regulatory actions involving light-emitting electronic products.

Differential Sensitivities:

Separate strains of the L5178Y mouse lymphoma cell line have been developed - H5178Y-R, which is highly tumorigenic, x-ray resistant, and UV-, heat, and drug-sensitive; and H5178Y-S, which is non-tumorigenic, x-ray sensitive, and UV-, heat- and drug-sensitive. These strains have been utilized to examine radiation and chemical mutagenesis, and to examine factors that relate mutagenesis with carcinogenetic potential. Data obtained with this test system have been used in regulatory actions involving light-emitting electronic products.

Neoplastic Transformation in Cell Cultures:

Methods have been developed that permit direct inspection of cell cultures to identify cells that have lost contact inhibition; that is, crowded cells will resume proliferation and will pile up in a growth pattern associated with cancer cell growth. The transformation assay has been used to examine radiation and chemical carcinogenesis; data from use of the system have been used in regulatory actions involving light-emitting electronic products and photosensitizing chemicals.

Viral Probes:

The use of viruses has been exploited for development of methods for cellular studies of acute toxicity. To date, it has been determined that the ability of cells to support replication of viruses closely parallels cell survival. The viral probes have been used to screen a group of photosensitizing chemicals, some phototherapeutic drugs, and a known carcinogen. The carcinogen use was associated with an enhancement of reactivation of cellular capacity to support viruses.

Cell Toxicity Reactions to Medical Devices:

Evidence is accumulating that some types of medical device materials react in the body and may undergo sufficient change to impair the function of the implanted device. Methods are being developed to examine materials placed in tissue cultures, with cell toxicity as an endpoint, to determine what changes are apparent in the material, and to examine the possibility that highly reactive states of certain metabolites of the cells may be associated with degradation of the material.

Biotechnology Probes:

Work is under way to develop DNA probes for use in toxicity testing. At present, analysis of DNA fragments resulting from restriction enzyme digestion of DNA are being used to detect abnormal genes. The fragments are being marked - hybridization of the fragment with specific DNA sequences, with viral and bacterial DNAs, and with RNA thus forming complexes that can be used either qualitatively or quantitatively to detect the presence of the substance of interest, or to locate and isolate the substance, or to observe its behavior during and subsequent to the application of a stressor of the test system.

Cellular Immunity:

Immunological defects are present in a number of human disorders that heighten sensitivity to radiation, and include ataxia telangiectasia, xeroderma pigmentosum, Bloom's syndrome, and systemic lupus erythematosus. Studies are underway to develop suitable cellular systems that can be used to study immune responses. Such systems include cell membrane modification (remodelling) so that the receptor areas change their functions in attaching molecules at the cell membrane surface; and the modification of the attaching molecules - modulation of antigens. In these studies, ELISA-like probes will be developed and used.

Cell Chemotaxis:

Macrophages, and in certain situations monocytes may respond to the presence of foreign substances and migrate to the site of the substance. Methods have been developed to examine the migration of macrophages across of porous membrane to a diffusable substance. Such recruitment of macrophages from implanted devices can present evidence of materials leeching from the device. Aspects of cell chemotaxis have been pursued in studies of ultraviolet and microwave effect. In neither case was chemotaxis clearly demonstrated, although cell migrations were observed.

Center for Food Safety and Applied Nutrition

Percutaneous Absorption Methods:

Testing is done to improve *in vitro* methods for measuring and predicting percutaneous absorption of cosmetics. Methods are being developed, using tissue culture media, to maintain viability of skin during *in vitro* studies.

Culture - *In Vitro* Cardiotoxicity:

Testing is done to improve and validate an *in vitro* system through the use of primary cell cultures of neonatal rat hearts for rapid and convenient evaluation of potential cardiotoxic agents on isolated cells from target organ tissue, and of the impact of nutrients on cell function and resistance to cardiotoxicity.

DNA Repair in Primary Rat Hepatocytes - Procedural Aspects:

Testing is done to identify and define several methodological aspects in the conduct of the DNA repair test with primary rat hepatocyte cultures in an attempt to standarize the test for uniformity and reproducibility between experiments with emphasis on weakly active agents and low doses of active substances.

Evaluation of *S*. *Typhimurium* Strain TA97A:

Testing is done to determine whether *Salmonella typhimurium* strain TA97A is more effective at detecting mutagens than strain TA1537.

In Vitro Transformation Method:

Testing is done to develop a metabolic activation system for *in vitro* transformation assay using BALB/3T3 cells.

In the evaluation of chemicals for carcinogenic or tumor promoting potential, *in vitro* transformation is unique in that the endpoint measured is the alteration of cell morphology. This assay can be used to identify chemicals not readily detected in mutagenesis assays. The cultured cells, however, do not have the capacity to metabolize all chemicals to their ultimate reactive forms. A noncytotoxic exogenous activation system is a critically needed component.

Cryopreserved BALB/3T3 Clone A31-1-1 cells will be used for the study. This clone has been characterized with respect to saturation density, cloning efficiency, doubling time and responses to model carcinogens. Effort will be directed toward the development of a metabolic activation system which can be incorporated into the *in vitro* transformation assay. Microsomal fractions will be prepared from rodents and tested first for their cytotoxicity then for their ability to activate selected chemicals to induce morphological transformation of the cells.

Biochemical Indices of *In Vitro* Developmental Toxicity:

The objective of this study is to evaluate interactions between nutritional status and exposure to natural toxicants on embryonic development. The rodent embryo culture system developed and standardizsed in previous fiscal years will be utilized in these studies.

Development of Biochemical Correlates of *In Vitro* Evaluation of Natural Toxicants:

The objective of this study is to develop biochemical methods for the *in vitro* evaluation of food related toxicants, specifically the use of a kidney explant system for the investigation of renal toxicants.

Biochemical Parameters - Macromolecular Biosynthesis:

Testing will develop and utilize sensitive methodology to study the subtle effects of natural toxicants on macromolecular metabolism in animal cells.

In Vivo and *In Vitro* Models for Dermal Toxicology:

This study will continue to investigate and improve *in vivo* and *in vitro* testing procedures intended to predict human skin irritation, sensitization and phototoxicity associated with exposure to cosmetic ingredients or products.

In Vivo and *In Vitro* Models for Ocular Toxicological Research:

The objective of this project is aimed at conducting *in vivo* and *in vitro* research to improve testing procedures for evaluating eye irritation and other adverse ocular effects.

Isolation of Foodborne Pathogens and Evaluation of Their Relationship to Human Disease:

Testing is designed to develop a specific serological assay for the *A. hydrophila* B-hemolysin (cytotoxic-enterotoxin) that is active in the rabbit ileal loop and suckling mouse assays. To identify virulence determinants of *A. sobria* and *A. caviae*.

Pathogenicity and Incidence of Foodborne Microbes:

Testing will develop, design, and implement new tests for foodborne microbial pathogens. Most of these tests will be based on DNA hybridization methods used to detect specific genes that are essential for microbial virulence. Effects will be made to develop synthetic deoxyribonucleotide hybridization probes for these genes.

Pathogenicity Surveillance Mechanisms and Incidence of Foodborne Microbes:

Objectives of this research are to purify the enterotoxin produced by *Yersinia pseudotuberculosis*, develop antibodies directed against it, and develop an immunological test to detect the enterotoxin.

Collaborate with Mr. Robert Becker, FDA, Dauphin Island, Alabama, on the screening of clinical isolates of *Plesiomonas shigelloides*. This screening will be performed in adult rabbit ileal loops and tissue culture models.

To isolate and characterize the factor(s) produced by *Vibrio cholerae*, strain CVD 105, that are responsible for the virulence of this microbe. This strain is devoid of both the vibrio cytolysin and cholera toxin, yet continues to produce diarrhea in human volunteers.

Pathogenicity and Incidence of Foodborne Microbes:

The study will identify the virulence factors produced by clinical isolates of *Campylobacter* species. To develop regulatory tests for the identification of pathogenic isolates from foods.

Campylobacter jejuni/coli cause between 5 and 10 percent of all diarrhea in the United States. This is more than the number of infections caused by *Salmonella* spp. and *Shigella* spp. combined. Since the mechanisms of pathogenicity for *Campylobacter* spp. are unknown, this study is designed to identify the responsible virulence factors.

Isolation of Foodborne Pathogens and Evaluation of Their Relationship to Human Disease:

Testing will develop reliable laboratory tests that will distinguish pathogenic from non-pathogenic strains of *V. vulnificus* and other opportunistic pathogens.

Enzymatic and Chemical *In Vitro* Methods to Evaluate the Protein Quality of Infant Formula:

The study objectives are to further expand the data base on the use of *in vitro* methods and to test the applicability of selected *in vitro* methods for determining the protein quality of infant formulas. An *in vitro* enzymatic digestion method tested in house predicted moderately well the digestibility component of protein quality.

Methodology Development - Mutagenic Testing:

The objective of this work is to evaluate and improve upon methods that have been devised for the detection of mutagenic and carcinogenic chemicals in certified colors.

Chemical/Instrumental methods for vitamin analyses: Methods are under study that would replace current bioassays for vitamins, e.g., the rat bioassay for vitamin D.

FDA Research Initiatives Which Will Replace or Refine Existing Tests

The following research activities are being or have been undertaken by FDA to develop new and appropriate biological tests which could replace or refine existing tests and reduce the number of animals currently used.

Center for Drugs and Biologics

Pyrogen Testing:

Guidelines are being written and will be completed within the next several months defining what a manufacturer must do to use the Limulus Amebocyte Lysate (in vitro) test to replace the rabbit pyrogen test (in vivo). Many manufacturers have already switched from the use of rabbits to the use of the horseshoe crab amebocytes for testing for endotoxin.

Plague Vaccine:

Over the past several years FDA has had a contract with the Department of Navy to develop a single, direct Enzyme Immunoassay (EIA) (in vitro) that can be used to standardize plague vaccine lots based on their content of the protective components. This assay would replace the bioassay (mouse test) that is presently the only means of calibrating plague vaccine. It appears that the in vitro test will replace the use of mice within the next year or two.

Diphtheria Antitoxin:

The tissue culture microtiter technique involving challenge of diluted diphtheria antitoxin with toxin in cell culture is being developed to replace the guinea pig (in vivo) test.

Rabies Vaccine:

Reagents and Techniques for in vitro potency testing of Rabies Vaccines using the Single Radial Immunodiffusion (SRID) Technique have been developed. These techniques are similar to those used for influenza virus potency testing and could lead to an improved rabies vaccine potency assays and eventual replacement of the standard NIH mouse potency test. An international collaborative study is being initiated and will involve control agencies and/or manufacturers from the following countries - United Kingdom, France, Germany and Canada.

Dr. **Guest**. Recognize that much of the activity that we have going on in the alternatives area has to do with substituting for safety and efficacy testing, so there is a large incentive in the drug industry and the food additives industry to move in those directions because of economics and public opinion.

We are not primarily a research group, as you know. Our research budgets are relatively small as compared to some of the other HHS agencies.

I can cite for you a number of examples where we have spent time and money in order to develop the quality control kinds of tests that would substitute for animal use. For instance, in several viral vaccines we are now using in vitro testing techniques for instance, for the inactivated polio vaccine, for the rabies vaccine, and for the hepatitis vaccine.

Another test that we routinely ask for from a drug sponsor are those things that would detect pyrogens or endotoxins in injectable drug products. The system for the horseshoe crab, ameba site test in lieu of the rabbit is coming to favor and is being widely used now.

We have developed model systems to test drugs for use in heart worm therapy in dogs so that we reduce the number of dogs needed for drug testing.

Mr. **Walgren**. You have submitted for the record a list of the research activities. If you asked the question, though—and I haven't seen that list, so I look forward to that—if you were asked a question, how many programs has the FDA initiated in the last 3 years in direct pursuit of alternatives, would there be a good answer to that?

Dr. **Guest**. I think the amount of dollars spent as relates to our research budget would be substantial, but I would have to furnish that for the record. I don't have it in front of me.

Mr. **Walgren**. Let me ask you if you would furnish that for record so that we can focus on those last 3 years to see what is developing. And that is the real question, whether there are some things happening that we can be encouraging and that you are obviously seeing before we see them.

Mr. Boehlert.

Mr. **Boehlert**. Dr. Willett, are NIH grants reviewed to insure that animal use is cut to a minimum?

Dr. **Willett**. Not directly—but I am going to answer, yes, in the following sense. That when a research proposal is reviewed by the peer review group examining it, they will look at the suitability of the systems that the investigator is proposing for use to obtain the objectives he says are worth obtaining. That will include, if he using an animal model, what one he is going to use, why he is going to use it, how many, et cetera. To the extent that that would be inappropriate in the eyes of his peers in the science community, it would definitely be reviewed.

Mr. **Boehlert**. Have you ever canceled any projects because of state and Federal regulations on animal research?

Dr. **Willett**. Have I?

Mr. **Boehlert**. Well, NIH?

Dr. **Willett**. Canceled research projects; yes. There was one case in point—you mean prior to review or——

Mr. **Boehlert**. When the award has already been made, a project is in process, you have reviewed the progress and made a determination that it should be canceled; have you ever done that?

Dr. **Willett**. I really can't answer that, but I am sure I could supply that to you. I could check back with my colleagues.

Mr. **Boehlert**. Dr. Rall, when you added parenthetically that you are not an expert on dairy cows, my ears perked up, because with your credentials, and your experience, and the fact that you are not an expert makes you an ideal candidate to run that program. We might get some common sense.

How close are we, Doctor, to finding an alternative to the Draize test?

Dr. **Rall**. I really don't know. Again, I just have not followed the Draize test. That is not an area of toxicological investigation that we have pursued.

Mr. **Boehlert**. Dr. Guest, would you be the person to ask?

Dr. **Guest**. My sense is that we are very close. And very close in science is maybe 4 or 5 years, sometimes, but that is close.

I think probably Dr. Goldberg is probably better equipped to answer the timing than I am, but I am encouraged by the progress.

Mr. **Boehlert**. You mentioned—you reported on that acute studies workshop, and if I read what you are saying correctly, you are not very enamored with the LD-50 test. You say FDA does not require the use of the LD-50 test. Do you do anything to discourage its use?

Dr. **Guest**. We have gone about very carefully expunging any words that have to with that kind of toxicity test from any guidelines or regulations that we have. And, in fact, I guess, the latest to go was in May of last year, when we stopped requiring it on some antitumor antibiotic batch testing.

What one does is explain in guidelines that these are the kinds of tests that are necessary, are certainly not the LD-50, and then the scientists as they review the protocols that the drug sponsors bring forward, look for signs of either excessive use of animals or excessive procedures in terms of humane care of animals. So, I think, probably, yes; and as well as every time we are asked, and any time anybody will listen, we tell them that the LD-50 is certainly not the precise kind of test that we need.

Mr. **Boehlert**. Would you say the use of animals in testing is on a decrease, leveled off, increasing, what do you think?

Dr. **Guest**. The numbers are not very precise, and you can't set many trends if you have to ask for numbers. But my impression, just having been in the business for awhile, is that the incentives are away from animal use.

Mr. **Boehlert**. Dr. Rall, you are nodding yes.

Dr. **Rall**. Unit and level are going down, I would guess. But again, that is sense, that is not hard data.

Mr. **Boehlert**. Dr. Willett, do you have the same sense?

Dr. **Willett**. I really don't know with the information that is available.

Mr. **Walgren**. If the gentleman would yield?

You look at NIH's budget, the amount that is being spent on research involving animals is going up. I would gather that the amount that we are able to identify for nonanimal research is extremely limited.

Dr. RALL. One comment; the NIH budget over the last 6 years in constant dollars, has been level.

Mr. WALGREN. But is that to again say that the amount spent on animal testing, the amount spent on projects which use animal testing has not gone up?

Dr. RALL. The amount has gone up. But it is my sense that you are buying the same amount of research, the same amount of animal testing. I don't think animal experimentation—I don't think it has gone down, but I don't think it has gone up either.

Mr. WALGREN. Has the amount of money in direct pursuit of alternatives increased?

Dr. WILLETT. Again, if we use in testing I would imagine, yes. If you look at the total package of the NIH portfolio, that category that doesn't use either mammals or human subjects has again remained essentially constant since 1977, if that is of any use.

Mr. BOEHLERT. Dr. Guest, one last question. On page 4, you say, our agencies or our Government are cooperating on animal welfare issues with other countries through organizations like the OECD. Are we the teacher, or are we the student?

Dr. GUEST. Oh, I think we are often the leader in those areas in developing harmonized guidelines, and requirements that would be acceptable around the world in testing. So I think quite often we are the teacher. We always learn; but we are obviously a leader.

Mr. BOEHLERT. That is good to hear. Thank you very much, no further questions.

Mr. WALGREN. Thank you, Mr. Boehlert.

What happens when budgets are reduced—and we had a reduction in April of this year—were the non-animal alternatives programs were reduced more than other programs were reduced, or was the reductions equal?

Dr. RALL. At least speaking for my institute, it was on a case-by-case basis. It was an attempt to determine whether or not each grant could take 5 or 10 percent decrease to make up for those that couldn't take any. I saw no trend either for or against the use of whole animals.

Mr. WALGREN. How about the other agencies involved?

Dr. WILLETT. In the case of the division, the reduction was the same in all the areas.

Mr. WALGREN. And how about FDA?

Dr. GUEST. I don't see any disproportionate cut, Mr. Chairman.

Mr. WALGREN. Do you have access to a nuclear magnetic machine, is it economical?

Dr. RALL. It is terribly expensive in terms of the first cost. It used to be known as nuclear magnetic resonance, but it has nothing to do with radioactivity, so its name is changing. It has to be in either a building with essentially no steel, or a constant steel frame.

Mr. WALGREN. Yes, I know it is expensive.

Dr. RALL. But we think in the long run it may be very economical once you get over the capital cost of buying it and installing it.

Mr. WALGREN. Have we bought it?

Dr. **Rall**. NIH has a number, and we have—NIH has at least one that is suitable for patients. We are buying two machines which are suitable for animals.

Mr. **Walgren**. Do we have them now. Are we running animals——

Dr. **Rall**. Oh, yes.

Mr. **Walgren**. That is good.

Dr. **Rall**. We would be glad to submit for the record some of the pictures, they are awful impressive.

Mr. **Walgren**. Dr. Rall, you are really on record as feeling the LD-50 test is a pretty—not so useful measure of measuring health hazards.

Dr. **Rall**. I would like to make one comment about the LD-50 test. I think it has gotten a bad rap today in a sense.

It was designed back in the days of tinctures and elixirs and pharmaceutical agents that were made by taking a plant and extracting it—digitalis for example, tincture of digitalis was the standard treatment for heart disease. Tincture of digitalis is a very uneven compound. Some plants had much more than others. And so you were faced with a very variable, but basically highly toxic medicine.

The LD-50 was designed to try to calibrate the toxicity of each batch, so that you could make it uniformly toxic when it went out to the pharmacist, and that is its real purpose. And for that reason you needed a kind of precision that I think we don't need anymore.

And the sort of range finding tests and so forth are quite adequate for today, use less animals. But in its time the LD-50 saved a lot of people from digitalis toxicity. But I agree that there is almost, almost no place for it.

Mr. **Walgren**. But yet the companies still use it. Even though you have eliminated it from your specific requirements, there seems to be a momentum out there that if somebody wants to have an application that would not be criticized, they went to a portion of it that says that we did it in LD-50 tests, and 50 of them died, or 50 of them didn't die.

Are there other positive steps that we are going to have to do to stop the inappropriate use of LD-50 by some testing systems?

Dr. **Guest**. I don't think we see that very often. In talking to all the centers in Food and Drug Administration, I ask that question, How often do you actually see the test? And the answer was, very, very seldom. But some companies still feel that they have to run that test to give some sort of a rank in toxicity, and perhaps and know about it in terms of protecting workers.

There are more precise acute toxicity tests, using much fewer animals that we normally see. I think that the move is definitely away from that, and there is certainly no requirement for that, Mr. Chairman.

Mr. **Walgren**. Dr. Rall, in the toxicity program, I gather "*x*" amount is allocated for testing, and then another portion of your budget would be for efforts to develop alternatives to testing, is that a fair——

Dr. **Rall**. Well, no, the budget is really developed from the bottom up. What are the opportunities in test method development; what are the list of compounds that have not been tested.

Mr. **Walgren**. Yes.

Dr. **Rall**. And we balance those, it is not——

Mr. **Walgren**. And in last year's budget, what was the percentage in development of testing, development of new methods of testing; if that is not an unfair question?

Dr. **Rall**. It was a little—in 1985 the total figure was $17 million out of about $70 million.

Mr. **Walgren**. $17 million out of $70 million?

From the congressional standpoint there are relatively few tools we have to work on moving that number, and one tool, I guess, is to try to structure offices—you don't have an office for the development of testing, do you, development of new methods of testing?

Dr. **Rall**. Yes, we have offices—we have people who are involved in testing. They tend to be one of two types, they are either interested in doing the test or they develop test methodology.

They are not segregated because they really work very well together, and it is important that they do. But many of our scientists in the toxicology testing and research program are devoted to developing new tests. This is what they like to do.

Mr. **Walgren**. Well, we are looking at 17 percent of the budget there and it is not organizationally under somebody who is feeling the responsibility of the development of new test methods?

Dr. **Rall**. It couldn't be, because the tests are so diverse, from tissue culture to measure to use, to measure renal function, simple tests for noninvasive test for neural behavior effects. You need to be in the discipline.

I think the fact that we have over the last 7 or 8 years, increased it percentagewise, indicates that the program is interested in as much emphasis as is scientifically available on developing new and alternative test methods.

Mr. **Walgren**. Well, certainly inasmuch as this seems to be an area that represents so much potential—Dr. Willett has been quoted in Science Magazine as saying that this is really the doorstep of a new theoretical biology—we want to push the frontiers as much as we can. I would like to encourage you in that direction, and indicate that when you take the actual measure, if you run the thermometer up the wall and ask, what percentage goes to that, and what percentage goes to that, it looks like the alternative development is underemphasized, let's put it that way, compared to the potential that it might have.

Dr. **Rall**. The decisions are very difficult. How would you decide between putting more money into a new test method, and testing a compound that many, many hundreds of thousands of people are exposed to that has never been tested? That is a difficult decision.

Mr. **Walgren**. Yes; I appreciate the difficulty.

Well, we would like to submit some questions to you for written submissions. We appreciate your being a resource to us, and want to encourage you in this area.

Thank you very much.

Dr. **Rall**. Thank you, sir.

Mr. **Walgren**. The last witness, Alan Goldberg, director for the Center for Alternatives to Animal Testing at Johns Hopkins.

Welcome, Dr. Goldberg, we appreciate your being here.

We will certainly include anything in writing you would like to submit in this exploration, and would love to hear your perspectives at this point.

Dr. **Goldberg**. Thank you, Mr. Chairman, members of the subcommittee, I have been asked to detail the accomplishments of the Johns Hopkins Center for Alternatives to Animal Testing, and my feelings about the future of in vitro toxicology. I am pleased to have this opportunity.

Mr. **Walgren**. Let me to ask you to pull that mike right in; I think it needs to be spoken right into before it will project to the back of the room.

Dr. **Goldberg**. I am Alan M. Goldberg, associate dean for research at the Johns Hopkins School of Hygiene and Public Health and professor and director of the Johns Hopkins Center for Alternatives to Animal Testing.

The center was established in 1981 by an enabling grant from the Cosmetic, Toiletries and Fragrance Association, a trade association representing approximately 200 companies with other major financial support provided by Bristol-Meyers Co., Exxon Corp., the Geraldine R. Dodge Foundation, and Amoco Corp., and others through individual support, and small support from around 30 to 40 other additional companies.

The goal of the center is to develop and disseminate appropriate basic scientific knowledge for innovative nonwhole animal methods to evaluate fully the safety of commercial and/or therapeutic products.

The center advisory board, currently consisting of 21 scientists, establishes policy, and conducts competitive review of investigator initiated grant applications. I have attached to my testimony a list of current projects.

The grants are generally funded for 1 year with continuation funding depending upon results and productivity. We hope that our grantees then apply to other sources for additional funds as well.

Voting members of the board are drawn from top universities throughout the Nation. Leading scientists from the center's industrial sponsors, government and the animal welfare movement serve as nonvoting members. And again, I have attached the roster of the membership to my testimony.

The center hosts an annual scientific symposium that each ear has established a new landmark in the development and implementation of alternatives to animal testing. The first symposium outlined problems impeding the search for alternatives.

The second produced a scientific consensus on short-term measures that could be taken to reduce animal use in acute toxicity testing. That consensus led to the actions by the U.S. Food and Drug Administration, and the U.S. Environmental Protection Agency encouraging that development. The meeting that we just heard referred to as the November 9 meeting.

The third meeting constituted a progress report on in vitro toxicology, with emphasis on potential alternatives to the controversial Draize eye test. The fourth symposium, held approximately a month ago, on April 14 and 15 of this year, concentrated on validation of in vitro methods, the final process in securing widespread acceptance of alternative tests. Proceedings of the symposia form a book series titled "Alternative Methods in Toxicology," published by Mary Ann Liebert, Inc.

The center distributes 17,000 of its newsletters—and, again, I have attached copies of two most recent newsletters to this testimony—to an international readership of scientists, corporate executives, government officials, animal welfare advocates, members of the news media and the general public.

[The information follows:]

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY - 5 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Mr. Doug Walgren
Chairman
Subcommittee on Science,
Research and Technology
Committee on Science and
Technology
Suite 2321
Rayburn House Office Building
Washington, D.C. 20515

Dear Mr. Chairman:

We are happy to have an opportunity to respond to your inquiry of April 24 concerning the use of animal tests by the Environmental Protection Agency (EPA) and the progress that has been made in reducing the use of animals for testing. Each topic will be answered in turn.

This Agency can require toxicological testing under two of its statutes, the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act. As part of our evaluation of chemicals concerning their potential to produce potential adverse health effects, we like to have data available following short-term and long-term exposures to the chemical, that is, estimates of acute toxicity and chronic effects. Occasionally, we have information on humans, but most often our assessments focus on data from experimental animals. We see animal test results as critical elements, because today, there are not many other scientifically acceptable means of predicting chemical safety. Even in light of this, we have taken several steps to decrease the use of animals.

1. Develop Consistent Protocols. Both EPA programs use the same test protocols for evaluating chemical hazards, and we have worked with other Federal regulatory agencies to devise consistent tests. On the international scene, we have actively contributed to developing similar toxicological tests within the Organization for Economic Cooperation and Development (OECD). In fact, it is the policy of this office to accept

studies done in accordance with OECD protocols, even if they differ somewhat from those used at EPA. In sum, the adoption of comparable approaches to toxicological testing results in considerable saving of animals both nationally and worldwide.

2. Reduce Numbers of Animals in Tests. In late 1984, EPA announced revised acute toxicity test guidelines. A three-step process was given. Initially, one reviews existing data on chemicals that are structurally related to the untested compound. In some cases enough information can be gleaned to obviate the need for any further testing. If not, we specify the use of a limit test, where a small number of animals is given a single, high dose of the chemical. If toxicity is not demonstrated, no further testing is needed. Only with those compounds showing toxicity, do we recommend further examination. In this case only three doses of the chemical are tested in small groups of animals. All toxic reactions, including death, are carefully observed including pathological examination at the end of the study. In this way we have maximized the amount of toxicological information while minimized the number of animals used to generate that information.

Although we recommend the above acute testing scheme, we discourage the employment of animals simply for the estimation of the median lethal dose (LD50). This position was clearly articulated in our announcement that accompanied the test guidelines and was sent to major toxicology societies, all major toxicology testing labs in the U.S. and other interested parties, including the animal rights groups and government agencies.

3. Alternatives to Mammalian Testing. Progress has been made in certain areas concerning the use of submammalian animals, cultures of mammalian cells and even plants to evaluate potential human health effects. The tests most widely used measure various genetic endpoints. They are used to help set priorities for testing chemicals, to evaluate data for carcinogenicity, and to evaluate the potential for heritable effects to future generations. In other cases cell preparations are used to study mechanism of toxic action and to evaluate chemical metabolism.

Finally, it is the position of EPA to incorporate test methods that reduce or replace whole animal testing as soon as they have been validated and found acceptable by the scientific community. Many different groups are making progress with alternative tests, especially as to possible replacements for the Draize eye test. We look forward to the fruits of these investigations.

In summary, then, EPA does rely on animal test results to make projections about public health. However, we have worked with others around the world to achieve consistent test protocols; we have clarified our position in regards to acute toxicity testing; and we incorporate alternatives to whole mammalian testing when they are readied by the scientific community.

Sincerely,

John A. Moore
Assistant Administrator
for Pesticides
and Toxic Substances

THE JOHNS HOPKINS CENTER FOR ALTERNATIVES TO ANIMAL TESTING

Vol. 4, No. 1 THE SCHOOL OF HYGIENE AND PUBLIC HEALTH WINTER 1985-86

The Center's First Four Years . . . and Beyond

A catalyst can speed up a chemical reaction by bringing the reactants closer together. The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) plays a similar role in the search for *in vitro* toxicity tests.

Since its inception in 1981, the CAAT has accelerated the quest by uniting the talents of top university researchers, the practical knowledge and financial support of industry, the authority of government regulatory agencies, and the concerns of the animal welfare movement.

A chemist would further define catalyst as a substance that remains unchanged by the reaction it stimulates. Here the CAAT differs. It has responded to the evolving needs of the nascent field of *in vitro* toxicology.

The Center's products: Books, symposia, research papers and newsletters have aided scientists in their search for alternatives to animal testing.

Having made advances toward tests of acute (short-term) toxicity, the Center is turning its attention to chronic (long-term) toxicity tests. It has placed more emphasis on validation, the process needed to prove that promising new methods really work. And the Center has established its own *In Vitro* Toxicology Laboratory to further speed the research effort and coordinate the transfer of technology from the Center to other labs in academia, government and industry.

This issue of the newsletter highlights the CAAT's progress during its first four years and explores what the future may hold for the Center and for *in vitro* toxicology. ☐

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CAAT Influential in Research, Public Information

By Alissa Swerdloff

During its first four years, the CAAT has been influential in two important areas.

Through its research program, the Center has demonstrated to the scientific community that a directed effort to find and develop alternatives can succeed.

Through its information program, the Center has improved the public's understanding of animal testing and potential alternatives.

The Research Program

The CAAT has shown that the investigator-initiated, peer-reviewed grant process can produce major advances in a goal-oriented program. This process has sped the development of alternatives and has helped to define the new discipline of *in vitro* toxicology.

By the end of fiscal 1[illegible], the Center will have dispersed more than $2 million for over 30 research projects. Already, the research program has produced more than 60 articles, book chapters, and symposium presentations, including 24 papers written for peer-reviewed scientific journals.

In more than a dozen of the CAAT-sponsored studies, scientists are examining *in vitro* methods to assess irritation and inflammation. This work includes research on alternatives to the Draize eye and skin tests.

In another dozen projects, researchers are looking for ways to evaluate chemicals' potential toxic effects on specific organs: the heart, lungs, liver, kidneys and nervous system.

The remaining projects are examining methods to detect compounds that produce birth defects, methods to reveal bacterial contaminants in food and drug products, and methods to assess cellular toxicity for broad use in toxicity testing.

Many *in vitro* methods are now also being studied by industrial concerns and regulatory agencies. This research gives industry toxicologists valuable hands-on experience in alternative methods and is an important step in winning the scientific community's acceptance of new methods for commercial use.

One CAAT-funded project that appears close to winning such acceptance is an *in vitro* test for botulism (see vol. 3, no. 2 of the newsletter). Developed by a Hopkins infectious disease researcher, it can replace a bioassay requiring up to 200 mice for each test.

The Center's success in research has bred copy CAATs. Within the last two years, Switzerland and West Germany have established centers for alternatives to animal testing, modeled on the Hopkins Center.

Scientists from Canada, England, Belgium, Norway, Australia, Israel, India, Japan and other countries have expressed strong interest in the CAAT's research program.

The Information Program

Through its publications and presentations, the CAAT is successfully drawing greater attention to alternatives and providing essential information to scientists, industry executives, government officials, animal protection advocates and the news media.

The CAAT newsletter has grown from an initial circulation of 1,000 to more than 15,000 and reaches key audiences internationally.

Alternative Methods in Toxicology, which presents the proceedings of the Center's annual symposia, is the only book series in its field.

CAAT director Alan M. Goldberg, Ph.D., and members of the Center's Advisory Board have counseled the U.S. government on major studies of alternatives to animal use. These studies were conducted over the last two years for Congress, the Air Force and the National Institutes of Health.

(continued on next page)

Shayne Gad, Ph.D.; Manager of Mammalian Toxicology; Allied Corporation

"I think it's pretty clear that the only reason there has been really significant activity and progress has been that the Center has provided a focus and the mechanism for a meaningful effort."

The Center's staff and Advisory Board members have participated in more than 100 seminars, symposia and presentations to scientific and lay communities.

As the result of a collaboration with the CAAT, the National Library of Medicine introduced "animal testing alternatives" as a new subject heading in all its new catalogs, periodicals and computer data bases (see vol. 3, no. 2). The new heading has improved communication on alternatives among scientists worldwide.

The CAAT also inspired the Library to create an annotated bibliography devoted to *in vitro* toxicology in *Tox-Tips*, a monthly periodical on toxicology testing.

The CAAT has reached millions of people through coverage in the news media. The Center's activities have been covered by newspapers, magazines, radio and television stations, and scientific, industry and animal-protection publications throughout the United States and in several other countries. Publicity has appeared in many large-circulation periodicals, including *The Wall Street Journal*, *The New York Times*, *The Los Angeles Times*, *The Chicago Tribune*, *USA Today*, *Newsweek* and *Business Week*.

The coming years should bring increased public interest, as the products of the Center's research gain acceptance in industrial testing laboratories. ☐

John McArdle, Ph.D., Director of Laboratory Animal Welfare, Humane Society of the United States

"In the animal welfare community, the response to the CAAT is very positive. The Center has clearly demonstrated that targeted development of alternatives can be done successfully. It is no longer possible to advocate the position that alternatives only come about through serendipity."

CAAT Establishes Lab

To supplement its research grant program, the CAAT has established its own *In Vitro* Toxicology Laboratory at the Johns Hopkins School of Public Health.

Under the leadership of the Center's associate director, John M. Frazier, Ph.D., the laboratory is conducting research in such areas as cellular toxicity, *in vitro* methods of evaluating chronic toxicity, and multiple-cell culture techniques that mimic interactions among cells in living animals.

The laboratory also will facilitate technology transfer from academia to industry. The lab's staff will train academic, regulatory and industrial scientists in the latest *in vitro* techniques. And the laboratory will coordinate inter-laboratory comparative studies to prove that the new methods really are better than live-animal testing.

The lab's research will focus on identifying the mechanisms of cellular toxicity. This knowledge will help scientists at the new lab and elsewhere to develop quantitative methods of analyzing cellular toxicity and, ultimately, to find alternatives to animal testing.

One research problem scientists will tackle is identification of biochemical responses of cells to poisons. If specific responses can be found and measured, they may enable scientists to study toxicity in one type of cell and then generalize the results to other types.

Another research team will try to grow a mixture of different types of cells in culture. This will permit scientists to study the interactions of different cells during poisoning. The current lack of research on multiple-cell cultures limits toxicologists' ability to apply the results of *in vitro* experiments to humans and other living animals.

The new lab also will investigate the feasibility of using *in vitro* techniques to study chronic toxicity. Until now, *in vitro* toxicologists have mainly concentrated their research on acute toxicity.

Scientists at the new lab will attempt to develop new tests based on the results of past CAAT-sponsored research on liver toxicity. The liver is a key player in the body's response to toxic chemicals.

Working from knowledge gained in their research on protein production and release in isolated liver cells, Hopkins toxicologists are collaborating with a private research lab to perfect a computer-based method of analyzing the proteins' biochemical "fingerprints."

This work should produce a quick and sensitive test that can be used not only to screen for potentially toxic chemicals but also to provide important information about mechanisms of poisoning.

Initial funding for the laboratory has been supplied by Allied Corporation, American Hospital Supply Corporation, Shell Companies Foundation and private donors. ☐

Symposia Advance *In Vitro* Toxicology

Academic, industry, and government scientists view the Center's annual symposium as *the* meeting in the field of *in vitro* toxicology. Participants say the gatherings not only serve as a showcase for cutting-edge research, they provide direction and cohesion to the new discipline.

The symposia's subject matter has followed a logical progression.

The first symposium, in May 1982, surveyed existing knowledge about alternatives to animal testing and outlined problems impeding the search for alternatives. A major focus was potential *in vitro* alternatives to tests for irritation and inflammation, such as the Draize eye test.

This symposium set the framework for the progress made in the Center's research program.

In May 1983, the second symposium produced a scientific consensus on short-term measures that could be taken to reduce animal use in acute toxicity testing. That consensus led to actions by the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency that encouraged industry to make reductions in the controversial classic LD_{50} test, which required the use of 40 to 200 animals per test.

Sidney Green, Ph.D.; Associate Director for Laboratory Investigations; Division of Toxicology; U.S. Food and Drug Administration

"The main impact of the CAAT is that it has brought alternatives to our attention and opened our eyes to options. It's been infectious — we're now doing more in vitro work ourselves."

Henry Spira; Coordinator; Coalition to Abolish the LD_{50} and Draize Tests

"The Center has been a leader. The credibility and prestige of Johns Hopkins has helped to legitimize the effort to develop alternatives . . . the Center has shown that good science can go hand-in-hand with reduction in animal use."

Alternatives methods adopted by industry provide information as useful as that obtained with the classic LD_{50} test. But they require as few as one-tenth as many animals.

Says FDA toxicologist Sidney Green, Ph.D.: "We definitely have seen a significant reduction in reliance on the classic LD_{50} test over the past two years. The Center for Alternatives to Animal Testing has played a role in this."

In October 1984, the third symposium constituted a progress report on *in vitro* toxicology, with emphasis on potential alternatives to the controversial Draize eye test. Many CAAT-sponsored scientists reported success in developing *in vitro* methods, with results that correlated well with those of live-animal tests.

This meeting was the subject of a cover story in *Chemical & Engineering News*, one of the most widely read and respected publications in the chemical industry.

The fourth symposium, scheduled for April 14 and 15, 1986, will concentrate on validation — the process of assessing the reproducibility, reliability and sensitivity of alternative tests compared with currently used animal tests. Regulatory agencies and commercial labs require extensive, scientifically sound validation studies to replace an animal test with an *in vitro* alternative.

Validation is the final stage in securing widespread acceptance of a particular alternative test or battery of tests.

(continued on next page)

Gary Ellis, Ph.D., Project Director for "Alternatives to Animal Use in Research, Testing, and Education", Office of Technology Assessment; U.S. Congress

"There are only two groups putting their money where their mouth is: the companies and industry associations supporting CAAT and those supporting the Rockefeller effort."

Proceedings of the CAAT symposia form a book series titled *Alternative Methods in Toxicology.* The first three volumes are now available from Mary Ann Liebert Inc., Publishers, 157 East 86th Street, New York, N.Y. 10028. ☐

Robert A. Scala, Ph.D., Senior Scientific Adviser for Environmental Health Sciences; Exxon Corporation

"The Center has leveraged its dollars. In many instances, it has been able to use its limited funds to add targeted studies on alternatives onto other research projects. And some researchers — initially funded by the CAAT — have been able to attract grants from other sources to continue their work on alternatives."

Bausch & Lomb Funds Draize Study

In September, Bausch & Lomb Inc. awarded the CAAT a $130,000 grant to conduct and publish a critical review of the Draize eye test and potential alternatives to it.

Under the grant, a group of independent scientists will review all available publications and research-in-progress to pinpoint the scientific and practical issues that must be addressed before Draize alternatives can be adopted by industry.

At a meeting in the spring, the authors of the review and scientists who have done research on the Draize test will evaluate and critique a draft of the review. After comment by this group, the final review will be written and published as a volume of the CAAT book series, *Alternative Methods in Toxicology.* ☐

Datebook

Dec. 9-10, Houston; Dec. 12-13, San Francisco; Dec. 16-17, San Diego: "Recombinant DNA Methodology Training Course." Contact: Roland M. Nardone, Ph.D., Center for Advanced Training in Cell and Molecular Biology, Catholic University of America, Washington, D.C. 20064. Telephone: 202-635-6161.

April 14-15

**"In Vitro Toxicology—
Approaches to Validation Methodology"**

Johns Hopkins
Center for Alternatives to Animal Testing
Fourth Annual Symposium
Baltimore, Md.

Deadline for Poster Abstracts: March 1

Contact:
Joan S. Poling
Center for Alternatives to Animal Testing
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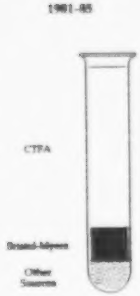
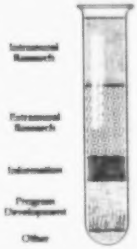
Financial Summary

Period: October 1,1981 through September 30, 1985





	1981-84	1984-85	1981-85
Revenues:			
Cosmetic, Toiletry and Fragrance Association	$ 916,000	$400,000	$1,316,000
Bristol-Myers Company	200,000	67,000	267,000
Geraldine R. Dodge Foundation	38,000	25,000	63,000
Exxon Corporation		50,000	50,000
Symposia (registration fees and sponsor's contributions)	50,411		50,411
Other Donations	71,162	29,832	100,994
Interest on Reserve Fund		6,000	6,000
TOTALS	$1,275,573	$577,832	$1,853,405
Expenses & Fund Balance:			
Intramural Research Grants	$ 384,786	$156,000	$ 540,786
Extramural Research Grants	368,058	214,460	582,518
Information Program (symposia, book series and newsletter)	125,790	51,000	176,790
Program Development and Administration	325,777	120,540	446,317
In Vitro Toxicology Laboratory		27,000	27,000
Reserve Fund			79,994
TOTAL			$1,853,405

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Dr. GOLDBERG. The eight-page, three times a year, publication, presents research results, highlights of symposia, bibliographies of scientific literature, information about the center and has become a resource for the development of in vitro toxicology.

The need to assess the safety of commercial and consumer products has been highlighted during the past several years. Technology and advances in chemistry have provided us with the opportunities of new products, of new formulations and of new entities.

It is widely accepted that in excess of 100,000 chemical compounds are currently in the market place for which we have little, if any, data on which to assign risk.

Additionally, the methods that have been accepted for the assessment of toxicity, tests like the LD–50 and eye testing in the rabbit are methods that were introduced about a half a century ago. These results, although generally not required by regulation, have become standard by history and tradition.

The increased awareness of potential hazards to chemicals has led to demand by consumers for more testing, for more effective testing, and for less expensive testing. This demand for increased testing has been counted by many for the decreased use of animals in testing strategies.

The request from the animal protection movement among others, confused the issues of basic biomedical research in routine product safety testing. Additionally, product development and screening were again issues that are not clearly understood by those requesting a decrease in animal usage. Some of the issues are addressing the OTA report that Dr. Ellis presented at the beginning of this hearing.

Coincidental with societal awareness and the need for product safety are major developments from the scientific standpoint in terms of providing new methods and new approaches in using a new biology to address these very real problems. Cell, organ and tissue culture methodologies, which were developmental in the basic biomedical research laboratory 15 or 20 years ago, were not even utilized in toxicological research 10 years ago.

In the late 1970's, a meeting was held of an international group on the use of tissue culture in toxicology. At that meeting, which took place in Holland, it became abundantly clear that a very small number of scientists internationally were using tissue culture in their basic research, but even less were thinking about the use of tissue culture in routine safety evaluation.

The term alternative is a relatively recent addition to the campaign literature of the animal-protection movement. And there is still widespread disagreement over its precise definition. Some organizations use the term to refer to only techniques that replace completely the use of animals in a particular area, for example, a computer model to predict LD–50 values.

However, others follow the definition developed by Russell and Burch in 1959; they defined an alternative as any technique that replaces the use of animals, that reduces the need for animals in a particular test, or that refines a technique in order to reduce the amount of suffering endured by the animal.

Thus, use of the up-down method to determine an acute toxicity value is an alternative to the classical LD–50 test because fewer animals are required. Today, these three Rs replacement, refinement, and reduction, represent the common definition of alternatives.

Mr. **Walgren**. What is the up-down method, I don't know that?

Dr. **Goldberg**. It is a methodology where an animal is given a dose which is thought to approximate the LD–50, if the animal does not die, then a higher dose is given by some log unit, or if the animal dies then a lower dose given to another animal, and by using somewhere in the neighborhood of 6 to 10 animals one can come up with an approximate number for that LD–50 value as defined by the classical LD–50.

Mr. **Walgren**. Thank you.

Dr. **Goldberg**. You are welcome.

The alternatives most commonly considered are cell, tissue and organ culture, computer modeling, and use of minimally invasive procedures and end-points that produce less stress. Although more and more toxicological research is being conducted in vitro, the potential of culture methods in toxicological evaluation and hazard assessment is only beginning to now be utilized and evaluated. This is the result of public pressure, the availability of new developments in basic biology, and maybe I should reemphasize that, it is the availability of new developments in basic biology, and increased recognition among scientists of the opportunities that in vitro methods provide for risk assessments.

However, the use of tissue methods must be developed and implemented cautiously in toxicology testing and hazard assessment. Obviously, a single culture cannot mimic the complex interactions of all cell types in the body, no matter how exquisite the experimental design. In vivo metabolism may be simulated to some extent, but not completely, and integrating functions such as immune reactions, and phagocytosis can only approached at this time.

In addition, culture systems are relatively static and the doses of the test chemical reaching the target system and the duration of contact may be the same as that which occurs in vivo. Culture methodology also presents physical problems regarding insoluble materials, stability of compounds, or biophysical effects of the test compounds.

On the other hand, culture technologies have great potential once investigators have acquired the background knowledge to ask highly focused and specific questions. The static nature of the culture methods is also an advantage in that the dose and duration of contact of a test chemical can be precisely determined.

Far less of the test chemical is required for in vitro investigations, therefore, one can easily set up replicate cultures and generate considerably more data in a short period of time.

One of the most exciting aspects of culture methodology in toxicology is that one can use human tissue. Such studies have been limited in the past because of the difficulty of growing and maintaining differentiated human cell types and culture. But technical problems are being steadily overcome.

Important developments in the last years include improvements in the quality control of the media in which we grow cells and the plastic ware in which grow cells, and improved quality control in the laboratories where better media formulations for the growth of normal cells that have specialized functions exist. For example, now it is possible to grow cells where the heart cells will continue to contract, and beat.

So these techniques have really led—that is the new biology that I referred to—previously have led to new opportunities in tissue culture that were really not available as recently as 4 or 5 years ago.

Previously, I classified in vitro methodology according to whether the approach is empirical, model development, or mechanistic. I would just like to emphasize two parts here of the prepared testimony.

The empirical approach to the development of methodology is problematic. The questions asked are generally not focused and correlations develop prior to fundamental understanding. Additionally, the reliability of predictions using such an approach tends to be uncertain. Should this be the case in the development of in vitro toxicological methods, we will unfortunately have provided supplementary testing strategies but not replacement testing strategies.

This will leave us with the dilemma attempting to use the in vitro methodologies without being able to rely on them.

Model development is another approach which utilizes systems that try to mimic the in vivo systems. Generally, the model system is neither complete nor faithful in all aspects of the system being modeled, but it tends to provide useful information if the data are not overinterpreted.

In those model systems where a single aspect of an an integrated response is examined, and the data are interpreted in that single system, this technique can provide meaningful inferences for the evaluation of chemical effects.

The mechanistic approach to the development of in vitro methodologies should be based on a thorough knowledge of the metabolism, kinetics, and biology of the system or species to be examined. If the metabolic pathways are understood, or if it is known that the parent compound produces a toxicological insult, then one can develop a system to examine the mechanisms by which the chemical or chemicals work.

That is one can examine the adverse chemical, the adverse chemical or physical effects that lead to significant functional loss in the tissue or system. This approach allows the in vitro system to be derived from the species under study. It provides a better understanding of chemical-biological interaction, and the consequences of that interaction.

Once a mechanism has been identified, it may then be possible to develop appropriate, interpretable, simple and reliable in vitro methodologies for toxicity testing. And it is important that we develop interpretable methodology.

From a scientific standpoint, the mechanistic approach is not only preferable but necessary. In vitro methods will be more acceptable and will develop rapidly when the knowledge base has advanced far enough to permit a focus on mechanisms.

However, this is a goal yet to be achieved and in the interim, we have to use whole animal approaches and to continue to develop other measures which will relay on tissue culture techniques and other in vitro methodologies that provide quick reproducible accurate methods for the assessment of safety.

Let me emphasize to eliminate animal testing at this time would constitute an abrogation of the toxicologist's responsibility to insure safety, and will pose a risk to human health that Government, industry and the public will find unacceptable.

If one traces the utilization of tissue culture and toxicity assessment, one can see remarkable progress having been made in just the first few years. The first early approaches to the use of in vitro tests looked at exclusion of dye as an attempt to measure viability and integrity of the cell membrane.

A second level which provided some greater degree of sensitivity looked at leakage of endogenous constituents of the cell in the medium. Most recently there has been interest in more functional aspects of the cell with the first attempts looking at the levels of specific components, then going to look at the rate of systhesis of those components, and most recently to the identification to specific alterations in those components.

These changes in approach, which seem small when described in a few sentences, required quantum leaps in our thinking and equally greater advances in the methodology available to us.

At this stage of the development of these more sophisticated systems, it is necessary to carefully evaluate the reproducibility, transferability and interpretability of the methods in product safety assessment. Significantly, correlative studies alone will not provide this information.

A coordinated and highly structured approach to validating many methods against each other, against our current methodology, and against the data available on human experience, will provide us with the next round of tests, this will provide us with an approach to the utilization of in vitro methodology in product safety evaluation and risk assessment.

Thank you, and I will be glad to answer any questions.

[The prepared statement of Dr. Goldberg follows:]

Testimony of Alan M. Goldberg, Ph.D. before the Committee on Science and Technology - May 6th, 1986.

Mr. Chairman and members of the committee, I have been asked to detail the accomplishments of the Johns Hopkins Center for Alternatives to Animal Testing and my feelings about the future of *in vitro* toxicology.

I am Alan M. Goldberg, Associate Dean for Research at the Johns Hopkins School of Hygiene and Public Health and Professor and Director of The Johns Hopkins Center for Alternatives to Animal Testing.

The Center was Established in 1981 by an enabling grant from the Cosmetic, Toiletries and Fragrance Association, a trade group representing approximately 200 companies with other major financial support provided by Bristol-Meyers Company, Exxon Corporation, the Geraldine R. Dodge Foundation, and Amoco Corporation.

The goal of the Center is to develop and disseminate appropriate basic scientific knowledge for innovative non-whole animal methods to evaluate fully the safety of commercial and/or therapeutic products.

The Center Advisory Board, currently comprising 21 scientists, establishes policy and conducts a competitive review of investigator initiated grant applications annually (list of current projects attached). Grants are generally funded for one year with continuation funding dependent on results and productivity. Voting members of the board are drawn from top universities throughout the nation. Leading scientists from the center's industrial sponsors, government and the animal welfare movement serve as non-voting members. (The membership roster is attached).

The Center hosts an annual scientific symposium that each year has established a new landmark in the development and implementation of alternatives to animal testing. The first symposium outlined problems impeding the search for alternatives. The second produced a scientific consensus on short-term measures that could be taken to reduce animal use in acute toxicity testing. That consensus led to actions by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency encouraging this development. The third meeting constituted a progress report on *in vitro* toxicology, with emphasis on potential alternatives to the controversial Draize eye test. The fourth symposium, held on April 14 and 15, 1986,concentrated on validation of *in vitro* methods, the final process in securing widespread acceptance of alternative tests. Proceedings of the symposia form a book series titled Alternative Methods in Toxicology, published by Mary Ann Liebert Inc. (157 East 86th Street, New York, N.Y. 10028).

The Center distributes 17,000 copies of its (free) newsletter (copies attached) to an international readership of scientists, corporate executives, government officials, animal welfare advocates, members of the news media and the general public. The eight-page, three-times-a-year publication presents research results, highlights of symposia, bibliographies of scientific literature, information about the Center and serves as a resource for the development of *in vitro* toxicology.

The need to assess the safety of commercial and consumer products has been highlighted during the last several years. Technology and advances in chemistry have provided us with the opportunities of new products, of new formulations and of new entities. It is widely accepted that in excess of 100,000 chemical compounds are currently in the market place for which we have little, if any, data on which to assign risk. Additionally, the methods that have been accepted for the assessment of toxicity, tests like the LD50 and eye testing in the rabbit are methods that were introduced about a half a century ago. These tests, although not required by regulation, have become standard because of history and tradition.

The increased awareness of potential hazards to chemicals has led to demand by consumers for more testing, for more effective testing and for less expensive testing. This demand for increased testing has been countered by many in the animal protection movement for the decreased use of animals in testing strategies. The requests from the animal protection movement confused the issues of basic biomedical research with routine product safety testing. Additionally, product development and product screening were again issues that are not clearly understood by those who are requesting a decrease in animal usage.

Coincidental with societal awareness and the need for product safety are major developments from the scientific standpoint in terms of providing new methods and new approaches using a new biology to address these very real problems. Cell, organ and tissue culture methodologies, which were developmental in basic biomedical research 15 or 20 years ago, were not even utilized in toxicological research 10 years ago.

In the late 1970s, a first meeting was held of an international group on the use of tissue culture in toxicology. At that meeting, it became abundantly clear that a very small number of scientists internationally were using tissue culture in their basic research, but even less were thinking about the use of tissue culture in routine safety evaluation.

The term alternative is a relatively recent addition to the campaign literature of the animal-protection movement, and there is still widespread disagreement over its precise definition. Some organizations use the term to refer only to techniques that replace completely the use of animals in a particular area, for example, a computer model to predict LD50 values. However, others follow the definition developed by Russell & Burch in 1959; they defined an alternative as any technique that replaces the use of animals, that reduces the need for animals in a particular test, or that refines a technique in order to reduce the amount of suffering endured by the animal. Thus, use of the up-down method to determine an acute toxicity value is an alternative to the classical LD50 test because fewer animals are required. Today, these three Rs represent the common definition of alternatives.

The alternatives most commonly considered are cell, tissue and organ culture, computer modeling, and the use of minimally invasive procedures and endpoints that produce less stress. Although more and more toxicological research is being conducted *in vitro*, the potential of culture methods in toxicological evaluation and hazard assessment is only now beginning to be utilized and evaluated. This is the result of public pressure, the availability of new developments in basic biology, and increased recognition among scientists of the opportunities *in vitro* methods provide for risk assessment.

However, the use of culture methods must be developed and implemented cautiously in toxicology testing and hazard assessment. Obviously, a single culture cannot mimic the complex interactions of all cell types in the body, no matter how exquisite the experimental design. *In vivo* metabolism may be simulated to some extent, but not completely, and integrating functions such as hormones, immune reactions, and phagocytosis can only be approached at this time. In addition, culture system is relatively static and the dose of the test chemical reaching the target system and the duration of contact may not be the same as those that occur in the in vivo test. Culture methodology also presents physical problems regarding the solubility, stability, and biophysical effects of the test compound.

On the other hand, culture techniques have great potential once investigators have acquired the background knowledge to ask highly focused and specific questions. The static nature of culture methods is also an advantage in that the dose and duration of contact of a test chemical can be precisely determined. Far less of the test chemical is required for *in vitro* investigations than in *in vivo* tests. Therefore, one can easily set up replicate cultures and generate more data in a shorter time.

One of the most exciting aspects of culture methodology in toxicology is that one can use human tissue. Such studies have been limited in the past because of the difficulty of growing and maintaining differentiated human cell types in culture. But technical problems are being steadily overcome. Important developments in the last years include improvements in the quality control of media and the plasticware provided by manufacturers, improved quality control in the laboratory, better media formulations for the growth of normal cells as well as for cells exhibiting specialized functions (e.g. heart cell contractility and melanin production by melanocytes), and improvements in cell separation and cloning techniques.

Previously, I classified *in vitro* methodology according to whether the approach is empirical, model development, or mechanistic.

The empirical approach to the development of methodology is problematic. The questions asked are generally not focused and correlations develop prior to fundamental understandings. Additionally, the results tend to be somewhat unpredictable. Should this be the case in the development of *in vitro* toxicological methods, we will unfortunately have provided supplementary testing strategies but not replacement testing strategies. This will leave us with the dilemma of attempting to use the *in vitro* methodologies without being able to rely on them.

Model development utilizes systems that try to mimic in vivo systems. Generally, the model system is neither complete nor faithful in all aspects of the system being modeled, but it tends to provide useful information if the data are not overinterpreted. In those model systems where a single aspect of an integrated response is examined and the data are interpreted in that single system, this technique can provide meaningful inferences for the evaluation of chemical effects.

The mechanistic approach to the development of *in vitro* methodologies should be based on a thorough knowledge of the metabolism, kinetics, and biology of the system or species to be examined. If the metabolic pathways are understood, or if it is known that the parent compound produces the toxicological insult, then one can develop a system to examine the mechanisms by which the chemical(s) work(s). That is, one can examine the adverse chemical or physical effects that lead to a significant functional loss in the tissue or system. This approach allows the *in vitro* system to be derived from the species under study. It also provides a better understanding of chemical-biological interaction and the consequences of that interaction. Once a mechanism has been identified, it may then be possible to develop appropriate, interpretable, simple and reliable *in vitro* methodologies for toxicity testing.

From a scientific viewpoint, the mechanistic approach is not only preferable but necessary. *In vitro* methods will be more acceptable and will develop rapidly when the knowledge base has advanced far enough to permit a focus on mechanisms. However, this is a goal yet to be achieved and in the interim, we have to use whole animal approaches and to continue to develop other measures which will rely on tissue culture techniques, and other *in vitro* methodologies that provide quick, reproducible accurate methods for the assessment of safety.

However, let me emphasize, to eliminate animal testing would constitute an abrogation of the toxicologist's responsibility to insure safety and will pose a risk to human health that government, industry and the public will find unacceptable.

If one traces the utilization of tissue culture in toxicity assessment, one can see remarkable progress having already been made in just the first few years. The first early approaches to the use of *in vitro* tests looked at exclusion of dye as an attempt to measure viability and integrity of the cell membrane. A second level which provided some greater degree of sensitivity looked at leakage of endogenous constituents of the cell into the medium, for example, enzymes. Most recently there has been interest in more functional aspects of the cell with the first attempts looking at the levels of specific components such as proteins, to the next level of advancement by studying the rate of protein synthesis, and most recently to the identification of alterations of specific proteins. These changes in approach, which seem small when described in a few sentences, required quantum leaps in our thinking and equally great advances in the methodology available to us.

At this stage of the development of these more sophisticated systems, it is necessary to carefully evaluate the reproducibility, transferability and interpretability of the methods in product safety assessment. Significantly, correlative studies alone will not provide this information. A coordinated and highly structured approach to validating many methods against each other, against our current methodology, and against the data available on human experience, will provide us with the next round of tests which will provide an approach to utilization of *in vitro* methodology in product safety evaluation and risk assessment.

Thank you, and I'll be glad to answer any questions.

AMG/ms

Mr. **Walgren**. Well, thank you very much, Dr. Goldberg.

How fast do you see those next steps coming on where you get validation and——

Dr. **Goldberg**. It is a process by which—for example, in the Draize test, which is one that has come up several times this morning, the methodology has moved to the point where there are now 30 methodologies, or thereabouts, that have been proposed as alternatives to the Draize test, proposed by those have developed them. Whether they are truly useful or not is yet to be determined.

Those tests have to validated in a highly coordinated fashion. The best estimates that one can have of developing the appropriate methodology, to transfer that technology to and from the development lab to a secondary lab, to identifying the specific compounds that one has to validate the methodology against, if it is a water soluble product, like a shampoo, it might be easy to transfer it. It is a grease like an axle grease, it might be much harder to to deal with that kind of problem.

To do those kinds of things we have estimated that it will take somewhere in the neighborhood of 4 or 5 years to go through a complete round of transferring the methodology, getting the laboratories set up so that they are all doing all of the same tests, and then looking at the data that they generate in what is coming known as the blind trial methodology, so that we have a very clear and precise picture as to what those methods tell us, and what they don't tell us.

Mr. **Walgren**. Are we at the point where we ought be having substantial effort invested governmentally, or through the mechanism of a center for alternative testing? We have centers for engineering design, and we are putting about $30 million into centers for engineering design at NSF. You have a center essentially funded by the——

Dr. **Goldberg**. It is private industry and individuals.

Mr. **Walgren**. Your center—do you fund individual research at that——

Dr. **Goldberg**. We fund within. We are really, I think, unique in that respect. The funds come to Johns Hopkins University, and then we fund research across both the United States, Europe, and Canada at this point, that will provide us with the best opportunities. We give very small grants out there, in the neighborhood of around $20,000 a year.

What has happened is that those grants are seed money, essentially, for the individuals to go to the federal system in the competitive process that the federal system uses. We use a similar kind of competitive peer review process.

Just three examples, one, that was referred to today was a grant at Michigan for skin. We funded that project and started it off in 1981, 1982 with our first round of funding. They then went to the Department of Defense, and have been funded by the Department of Defense since then, for considerably more on a yearly basis.

At the University of San Franciso, we funded the development of an artificial barrier to resemble a penetration, that would allow one to study the penetration of a material through the skin. We funded that for one year. During that year that individual got enough to data and went to the Environmental Protection Agency, and they have been funded through the Environmental Protection Agency.

The center itself has applied to the grant system that Dr. Rall referred to in his presentation. So the only thing is that we have used the funds provided by industry to attempt to develop a larger funding base through the Federal Government by competitive peer reviewed grants.

Mr. **Walgren**. How many of your grants go on to get that kind of other source funding, what percentage?

Dr. **Goldberg**. We haven't tried at this early stage systematically collecting that data. I sat down and wrote those out as the presenters were doing that as the questions came up. My guess is that we are probably in the neighborhood of most of our people being funded by the Federal Government before they ever come to us, and they are doing an additional thing on their Federal grants.

Others use that additional thing to go back to the Federal Government; so I really don't have a specific number.

Mr. **Walgren**. Would it be 70 percent, or thereabouts, that has a sort of reinforcing funding mechanism coming out of the Federal Government?

Dr. **Goldberg**. It is probably lower than that because we ourselves only refund about 70 percent. So my guess is that the same 70 percent would be competitive with the Federal Government, and probably in the neighborhood of 40 percent, or thereabouts, have probably attempted it; but that is really a guess.

Mr. **Walgren**. What is your view of what would happen if you had a greater focused investment by the Federal Government through some kind of center of set aside program, or something like that?

Dr. **Goldberg**. One of the points that I tried to make, and will try to make again, is that what this area needs most is very fundamental research; it is an applied area of using the basic biology that we generate, and then developing a method from that, and to try to develop the method from scratch is developing it without the substantial underpinning and solid scientific base that it needs.

Mr. **Walgren**. And is that the empirical or——

Dr. **Goldberg**. That is the empirical approach that I sort of referred to. Others have classified it in jest as one has the carnation test, where if you took the compound and put it into the carnation, and if the carnation died, the material was toxic. If that gave you 100 percent correlation, that would be very nice. But you have no faith in using that test to predict safety for other animals or other humans.

Mr. **Walgren**. So you would certainly favor the multiple small research grant proposal approach as opposed to the large focused institution like the Rockefeller University, in vitro laboratory?

Dr. **Goldberg**. Again, that is not the—the Rockefeller University is a laboratory project which is quite good, and they have done very nice things in developing alternatives to the Draize eye test. It is a focused program. I think that is fine.

We have taken a very different approach in trying to develop alternatives to other areas, and initially, in fact, in the first three years we did not work at all on the Draize eye test as an alternative, only during our fourth year did we begin that activity.

Mr. **Walgren**. Can you see the impact on FDA of the consensus agreements that were developed in your conference?

Dr. **Goldberg**. There are several levels at which I think I have seen that. Literally whenever I have spoken to large groups at the FDA or the EPA, there is a very respective audience. I tend to suspect that most industries at this point, although I can't confirm that, where they are submitting data that does not require the LD-50 by regulation, supply data obtained it by, either the limit test, or the by the up-down method. So that has already happened to a great degree. And that the classical LD-50 is a test that has passed its time and is really not used, except where absolutely required.

Mr. **Walgren**. It might not be absolutely required in the abstract, but by regulation?

Dr. **Goldberg**. Well, there are certain regulations that I am aware of that do require the LD-50, in the classical sense. And their submissions, I believe, are supplied with the classical LD-50 and appropriate number of animals.

Mr. **Walgren**. Yes.

If you were writing those regulations from scratch would you be requiring the LD-50 in those instances?

Dr. **Goldberg**. No, I would not. However, I think I should state that acute toxicity testing in some way is very necessary. The LD-50 by itself is something that as Dr. Rall, I think, adequately pointed out, was the first approach at standardization of toxicology, going back approximately 60 years ago, and that tended to hang on in summariness.

Mr. **Walgren**. Can you make any reasonable projection at where you would expect this area to be in 20 years?

Dr. **Goldberg**. That is a long-term look. I would suspect, within 20 years, because of the changes that I have seen over the first 4 years, where in vitro methodology will be the first tier of testing, routinely, and that in only very specific areas will animal testing then follow where additional information is needed. As it is now, compounds, for example, in Draize eye testing are done in tiered ways, so that if one knows that the compound is corrosive, it is no longer, I don't believe, put into a rabbit eye. It is identified as a corrosive.

The same goes on for other areas as well. So that there has been a changing sensitivity instead of just rote focus on standard operating procedures where there is a greater sensitivity in developing approaches that do minimize pain.

Mr. **Walgren**. But if you had in vitro testing as the first tier, very broadly, could you make an estimate of what the economic benefit or present cost avoided would be in the the, I don't know, the pharmaceutical industry, or whatever industries are involved?

Dr. **Goldberg**. I do not have that kind of data available, nor do I know anybody that really does.

Mr. **Walgren**. Is it a large number?

Dr. **Goldberg**. It is clear that it would be a large number, once there were good methodologies available. I know that in the area of teratology testing, whole animal, which is the production of looking at fetal effects, an animal study, probably costs in the neighborhood of $50,000 to $70,000. The current in vitro approach to that, which is being developed, which does not give anywhere near the same amount of information but is a very good first tier is about one-tenth of that approach; one-tenth of that price. So there is clearly an economic incentive.

Additionally, there are factors like to house animals is very expensive because it is people and it is space, and this becomes much less in terms of in vitro methodology.

Mr. **WALGREN**. I wonder if there is some way that an estimate could be made about the potential avoidable costs, and then the question would be to measure whatever our present investment in pursuit of that cost reduction is. Now, I realize it is——

Dr. **GOLDBERG**. You are way outside of my expertise.

Mr. **WALGREN**. I wonder where that might be; it might lie in OTA maybe?

Dr. **GOLDBERG**. They did have a section on the economic impact.

Mr. **WALGREN**. It would be interesting.

But obviously there is tremendous potential here for change and you see it moving?

Dr. **GOLDBERG**. I am exceedingly optimistic, but as I probably came across I am exceedingly cautious. I feel that if we try to implement methodology that is really not there, we will actually slow down the process rather than speed it up, because it will have lost any form of confidence that we can build up by developing different methodology.

Mr. **WALGREN**. Well, I think it is fair to say that everyone is sensitive to that, and that the effort that has mainstream support in the Congress is one that would not violate that sense of caution, and certainly would look to develop the progress before we moved away from present efforts that do give assurance and also knowledge.

We appreciate your being a resource to us. I want to say again that this is a sort of a forum for ongoing discussion, and so we expect a development.

In fact, I am thinking that one question that we didn't ask of Dr. Rall—was it Dr. Rall or Dr. Willett on the plan that Congress asked for that is due in October?

Dr. Willett.

Is Dr. Willett still here?

Maybe you can come back up, Dr. Willett, if you would?

The question is where are we on the plan that the Congress asked for that is due in October with respect to alternatives and with respect to training of people to broaden the use of alternatives?

Dr. **WILLETT**. The implementation plan was formulated and processed. There are several committees in the stage of organization to pull the pieces of information that are necessary to really produce an adequate response to that requirement.

Mr. **WALGREN**. Is it a complicated array of committees, or——

Dr. **WILLETT**. The idea is two, plus the BID directors committee that was mandated in the section of the law.

Mr. **WALGREN**. BID, what is that?

Dr. **WILLETT**. The Bureaus, Institutes, and Divisions of NIH were to have their directors as participants in the committee that was to aid the director of NIH in developing this plan.

Mr. **Walgren**. Can you tell anything about the initial work of those committees?

Dr. **Willett**. So far it is in process, it is under development, there is nothing that—there is no product at this point.

Mr. **Walgren**. But there will be by October, is that the feeling?

Well, we certainly are going to be more than interested in that. I am sure we will have a hearing right around that time in hopes of exploring how far you have come on that project.

We hope there will be other developments that we can talk about. So we would like to encourage you and know that we will be coming along right behind you.

Dr. **Goldberg**. We welcome that.

Mr. **Walgren**. Dr. Goldberg, thank you for your participation in this; we look forward to talking to you about it in the future.

Dr. **Goldberg**. Thank you very much.

[Whereupon, at 12:40 p.m., the hearing adjourned.]

APPENDIX

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY - 5 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Mr. Doug Walgren
Chairman
Subcommittee on Science,
Research and Technology
Committee on Science and
Technology
Suite 2321
Rayburn House Office Building
Washington, D.C. 20515

Dear Mr. Chairman:

We are happy to have an opportunity to respond to your inquiry of April 24 concerning the use of animal tests by the Environmental Protection Agency (EPA) and the progress that has been made in reducing the use of animals for testing. Each topic will be answered in turn.

This Agency can require toxicological testing under two of its statutes, the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act. As part of our evaluation of chemicals concerning their potential to produce potential adverse health effects, we like to have data available following short-term and long-term exposures to the chemical, that is, estimates of acute toxicity and chronic effects. Occasionally, we have information on humans, but most often our assessments focus on data from experimental animals. We see animal test results as critical elements, because today, there are not many other scientifically acceptable means of predicting chemical safety. Even in light of this, we have taken several steps to decrease the use of animals.

1. Develop Consistent Protocols. Both EPA programs use the same test protocols for evaluating chemical hazards, and we have worked with other Federal regulatory agencies to devise consistent tests. On the international scene, we have actively contributed to developing similar toxicological tests within the Organization for Economic Cooperation and Development (OECD). In fact, it is the policy of this office to accept

studies done in accordance with OECD protocols, even if they differ somewhat from those used at EPA. In sum, the adoption of comparable approaches to toxicological testing results in considerable saving of animals both nationally and worldwide.

2. Reduce Numbers of Animals in Tests. In late 1984, EPA announced revised acute toxicity test guidelines. A three-step process was given. Initially, one reviews existing data on chemicals that are structurally related to the untested compound. In some cases enough information can be gleaned to obviate the need for any further testing. If not, we specify the use of a limit test, where a small number of animals is given a single, high dose of the chemical. If toxicity is not demonstrated, no further testing is needed. Only with those compounds showing toxicity, do we recommend further examination. In this case only three doses of the chemical are tested in small groups of animals. All toxic reactions, including death, are carefully observed including pathological examination at the end of the study. In this way we have maximized the amount of toxicological information while minimized the number of animals used to generate that information.

Although we recommend the above acute testing scheme, we discourage the employment of animals simply for the estimation of the median lethal dose (LD50). This position was clearly articulated in our announcement that accompanied the test guidelines and was sent to major toxicology societies, all major toxicology testing labs in the U.S. and other interested parties, including the animal rights groups and government agencies.

3. Alternatives to Mammalian Testing. Progress has been made in certain areas concerning the use of submammalian animals, cultures of mammalian cells and even plants to evaluate potential human health effects. The tests most widely used measure various genetic endpoints. They are used to help set priorities for testing chemicals, to evaluate data for carcinogenicity, and to evaluate the potential for heritable effects to future generations. In other cases cell preparations are used to study mechanism of toxic action and to evaluate chemical metabolism.

Finally, it is the position of EPA to incorporate test methods that reduce or replace whole animal testing as soon as they have been validated and found acceptable by the scientific community. Many different groups are making progress with alternative tests, especially as to possible replacements for the Draize eye test. We look forward to the fruits of these investigations.

In summary, then, EPA does rely on animal test results to make projections about public health. However, we have worked with others around the world to achieve consistent test protocols; we have clarified our position in regards to acute toxicity testing; and we incorporate alternatives to whole mammalian testing when they are readied by the scientific community.

Sincerely,

JAMoore

John A. Moore
Assistant Administrator
for Pesticides
and Toxic Substances